Methods for Testing Immunological Factors

Martin Braddock

Contents

Methods for Testing Immunological Factors

In Vitro Methods

Inhibition of Histamine Release from Mast Cells

Purpose and Rationale

Hypersensitivity reactions can be elicited by various factors: either immunologically induced, i.e., allergic reactions to natural or synthetic compounds mediated by IgE, or non-immunologically induced, i.e., activation of mediator release from cells through direct contact, without the induction of, or the mediation through immune responses. Mediators responsible for hypersensitivity reactions are released from mast cells. An important preformed mediator of allergic reactions found in these cells is histamine. Specific allergens or the calcium ionophore 48/80 induce release of histamine from mast cells. The histamine concentration can be determined with the *o*-phthalaldehyde reaction.

Procedure

Preparation of Mast Cell Suspension

Wistar rats are decapitated and exsanguinated. Fifty ml of Hank's balanced salt solution (HBSS) is injected into the peritoneal cavity, and following massage of the body, the abdominal wall is opened. The fluid containing peritoneal

M. Braddock (✉)
Global Medicines Development Respiratory Projects, AstraZeneca R&D, Cheshire, England, UK
e-mail: martin.braddock@astrazeneca.com

F.J. Hock (ed.), *Drug Discovery and Evaluation: Pharmacological Assays*,
DOI 10.1007/978-3-319-05392-9_45

cells is collected in a centrifuge tube and centrifuged at 2,000 rpm. The cells are resuspended in HBSS. Then the cell suspension is brought to a final concentration of 10^5 mast cells/100 µl.

Test Compound Administration and Induction of Histamine Release

1 ml test drug (concentration range between 10^{-4} and 10^{-8} Mol) is added to the mast cell suspension (10^5 cells/100 ml) and the mixture is incubated at 37 °C for 15 min. The cells are made up to a volume of 3 ml with HBSS, an equal volume of calcium ionophore (10^{-6} g/ml), compound 48/80, or specific allergen is added. The suspension is incubated at 37 °C for 30 min followed by centrifugation at 2,500 rpm.

The Following Control Solutions Are Needed

- *Spontaneous histamine release*: contains only mast cells and solutions used to determine the baseline
- *Histamine release*: contains mast cells and solutions and calcium ionophore (10^{-6} g/ml)
- *Test compound control*: contains solutions and test compound to test the compound for native fluorescence
- *Solution control*: contains only solutions used in the test to determine the baseline

Extraction of Histamine

One ml of the top layer is transferred to a tube containing 300 mg NaCl and 1.25 ml butanol. The sample is alkalized to extract the histamine into butanol by adding 1 ml 3 N NaOH. Following mechanical shaking, the sample is centrifuged for 5 min. One ml of the top layer (butanol) is pipetted into a 5-ml tube containing 2 ml of n-heptane and 0.4 ml of 0.12 N HCl. The tube is mixed by inverting it several times. Following separation into aqueous and organic phases, 0.5 ml of the aqueous phase is transferred to another tube.

Induction of *o*-Phthalaldehyde Complexing Reaction

To each sample, 100 µl 1 N NaOH is added under constant stirring immediately followed by administration of 100 µl 0.2 % *o*-phthalaldehyde solution. After 2 min, the *o*-phthalaldehyde complexing reaction is stopped by addition of 50 µl 3 N HCl.

Determination of Histamine Release

The total sample is transferred to an autosampler vial, and the histamine concentration is determined by a fluorescence detector (using excitation and emission wave lengths of 350 and 450 nm, respectively).

Evaluation

Percent histamine release (hist. rel.) can be expressed by the following formula:

$$\frac{\text{Sample hist. rel.} - \text{Spontaneous hist. rel.}}{100\%\ \text{hist. rel.} - \text{Spontaneous hist. rel.}} \times 100$$

The statistical evaluation is carried out using the Student's *t*-test (comparison of 100 % control to experimental group).

Critical Assessment of the Method

Disodium cromoglycate has been reported to inhibit the release of histamine and the degranulation of rat mast cells (Orr and Cox 1969; Orr et al. 1971; Johnson and Bach 1975; Church and Young 1983). However, this effect of disodium cromoglycate and its analogues does not parallel the clinical efficacy (Kay et al. 1987).

Modifications of the Method

Johnston et al. (1978) studied the increased superoxide anion production by immunologically activated and chemically elicited macrophages.

Flint et al. (1985) found a significant inhibition of histamine release by disodium cromoglycate in human mast cells recovered by bronchoalveolar lavage.

Ali et al. (1985) investigated the histamine release from rat peritoneal mast cells, human basophil and neutrophil leukocytes, and mast cells from mesentery of the lung and heart of rats and guinea pigs by the skin irritating constituents thapsigargin and thapsigargicin from the resin of the umbelliferous plant *Thapsia garganica*.

Eady (1986) studied the reactivity of mast cells in bronchoalveolar lavage fluid of macaques repeatedly infected with *Ascaris suum*.

Wells et al. (1986) compared release of histamine, LTC_4, and PGD_2 from primate bronchoalveolar mast cells with that of rat peritoneal mast cells.

The release of β-hexosaminidase from mouse or rat bone marrow-derived mast cells and from rat peritoneal mast cells was studied by Broide et al. (1986).

Peretti et al. (1990) recommended flow cytometry to investigate mast cell degranulation. Peptides, including substance P and bradykinin analogues, release histamine from human skin mast cells (Lawrence et al. 1989).

Williams et al. (1991) studied the vancomycin-induced release of histamine from rat peritoneal mast cells and a rat basophil cell line (RBL-1).

Kase et al. (2009) studied the inhibitory action of roxithromycin on histamine release in mast cells and Yazid et al. (2013) provided further support for antiallergic activity of chromones.

A sensitive colorimetric assay for the release of tryptase from human lung mast cells in vitro has been described by Lavens et al. (1993).

References and Further Reading

Ali H, Brøgger Christensen S, Foreman JC, Pearce FL, Piotrowski W, Thastrup O (1985) The ability of thapsigargin and thapsigargicin to activate cells involved in the inflammatory response. Br J Pharmacol 85:705–712

Bartlett RR, Dimitrijevic M, Mattar T, Zielinski T, Germann T, Rüde E, Thoenes GH, Küchle CCA, Schorlemmer HU, Bremer E, Finnegan A, Schleyerbach R (1991) Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of auto-immune disorders and reactions leading to transplantation rejection. Agents Actions 32:11–21

Broide D, Marquardt D, Wasserman S (1986) Effect of nedocromil sodium and sodium cromoglycate on connective tissue and bone marrow derived mast cells: acute and chronic studies. Eur J Respir Dis 69(Suppl 147):196–198

Church MK, Young KD (1983) The characteristics of inhibition of histamine release from human lung fragments by sodium cromoglycate, salbutamol and chlorpromazine. Br J Pharmacol 78:671–679

Eady RP (1986) The pharmacology of nedocromil sodium. Eur J Respir Dis 69(Suppl 147):112–119

Flint KC, Leung KBP, Oearce FL, Hudspith BN, Brostoff J, Johnson N (1985) Human mast cells recovered by bronchoalveolar lavage: their morphology, histamine release and the effects of disodium cromoglycate. Clin Sci 68:427–432

Johnson HG, Bach MK (1975) Prevention of calcium ionophore-induced release of histamine in rat mast cells by disodium cromoglycate. J Immunol 114:514–516

Johnston RB, Godzik CA, Cohn ZA (1978) Increased superoxide anion production by immunologically activated and chemically elicited macrophages. J Exp Med 148:115–127

Kase K, Hua J, Yokoi H, Ikeda K, Nagaoka I (2009) Inhibitory action of roxithromycin on histamine release and prostaglandin D_2 production from β-defensin 2-stimulated mast cells. Int J Mol Med 23:337–340

Kay AB, Walsh GM, Moqbel R, MacDonald AJ, Nagakura T, Carroll MP, Richerson HB (1987) Disodium cromoglycate inhibits activation of human inflammatory cells in vitro. J Allergy Clin Immunol 80:1–8

Lavens SE, Proud D, Warner JA (1993) A sensitive colorimetric assay for the release of tryptase from human lung mast cells in vitro. J Immunol Methods 166:93–102

Lawrence ID, Warner JA, Cohan VL, Lichtenstein LM, Kagey-Sobotka A, Vavrek JR, Stewart JM, Proud D (1989) Induction of histamine release from human skin mast cells by bradykinin analogs. Biochem Pharmacol 38:227–233

Orr TSC, Cox JSG (1969) Disodium cromoglycate, an inhibitor of mast cell degranulation and histamine release induced by phospholipase A. Nature 223:197–198

Orr TSC, Hall DE, Gwilliam JM, Cox JSG (1971) The effect of sodium cromoglycate on the release of histamine and degranulation of rat

mast cells induced by compound 48/80. Life Sci 10:805–812
Peretti M, Nuti S, Parente L (1990) Investigation of rat mast cell degranulation using flow cytometry. J Pharmacol Methods 23:187–194
Riley PA, Mather ME, Keogh RW, Eady RP (1987) Activity of nedocromil sodium in mast-cell-dependent reactions in the rat. Int Arch Allergy Appl Immunol 82:108–110
Siriganian RP (1976) Histamine release and assay methods for the study of human allergy. In: Rose NR, Friedman H (eds) Manual of clinical immunology. American Society of Microbiology, Washington, pp 603–615
Skolfitsch G, Saria A, Holzer P, Lembeck F (1981) Histamine in tissue: determination by high-performance liquid chromatography PLC condensation with *o*-phthalaldehyde. J Chromatogr 226:53–59
Wells E, Jackson CG, Harper ST, Mann J, Eady RP (1986) Characterization of primate bronchoalveolar mast cells. II. Inhibition of histamine, LTC_4, and PGD_2 release from primate bronchoalveolar mast cells and a comparison with rat peritoneal mast cells. J Immunol 137:3941–3945
Williams PD, Laska DA, Shetler TJ, McGrath JP, White SL, Hoover DM (1991) Vancomycin-induced release of histamine from rat peritoneal mast cells and a rat basophil cell line (RBL-1). Agents Actions 32:217–223
Yazid S, Sinniah A, Solito E, Calder V, Flower RJ (2013) Anti-allergic cromones inhibit histamine and eicosanoid release from activated human and murine mast cells by releasing annexin A1. PLoS One 8:e58963

Mitogen-Induced Lymphocyte Proliferation

Purpose and Rationale

Cultured lymphocytes can be stimulated to a proliferative response and to DNA synthesis by various mitogens. Measurement of DNA synthesis can be accomplished by pulse-labeling the culture with tritiated thymidine (^{3}H-thymidine), a nucleoside which is incorporated into the newly synthesized DNA. Immunomodulating properties can be detected either by pretreatment of the animals in vivo or by adding the test drug to the cultured lymphocytes.

Procedure

Mice of NMRI strain weighing 18–20 g or rats of Lewis strain weighing 180–200 g are used.

Materials

Sheep red blood cell (SRBC)-specific antigen and/or the following mitogens:

- Lipopolysaccharide 10–0.1 μg/ml.
- Dextran sulfate 30–7.5 μg/ml.
- Phytohaemagglutinin 0.5–0.12 % stock solution.
- Concanavalin A 0.5–0.12 μg/ml.
- As standards, levamisole, cyclosporine A, prednisolone, or leflunomide are used.

Ex Vivo

Animals receive the test compound once a day for 5 days. Thereafter, they are sacrificed, spleens are removed, and a single cell suspension of 5×10^6 cells/ml is prepared. Mitogens are titrated (four replicates/group) in 0.1 ml/well and 0.1 ml of the cell suspension is added. Plates are incubated at 37 °C in 5 % CO_2 in air for 48–60 h and for another 8 h after addition of 0.25 μC ^{3}H-thymidine per well. Cells are harvested on glass fiber filters, and after drying the degree of radioactivity is determined using a β-counter.

In Vitro

Animals are sacrificed and their spleens removed. A single cell suspension of 10^7 cells/ml is prepared and 0.05 ml placed in each microtiter well (four replicates/group). Then the test compound (four times concentrated) is added in 0.05 ml. At last 0.1 ml of the double concentrated mitogen is added. Plates are incubated and processed as described above.

Evaluation

Stimulation index = proliferation ratio according to positive control, either with or without mean

spleen weight. Statistical evaluation is carried out using the Student's *t*-test (comparison of positive and/or negative control to experimental group).

References and Further Reading

Bartlett RR (1986) Immunopharmacological profile of HWA 486, a novel isoxazol derivative-II. in vivo immunomodulating effects differ from those of cyclophosphamide, prednisolone, or cyclosporin A. Int J Immunopharmacol 8:199–204

Delgado IF, Paumgartten FJR (2014) Effects of *Euphorbia milii* latex on mitogen-induced lymphocyte proliferation. Rev Bras Pl Med Camp 16:107–111

di Padova FE (1989) Pharmacology of cyclosporine (Sandimmune) V. Pharmacological effects on immune function: in vitro studies. Pharmacol Rev 41:373–405

Elves MW (1972) The lymphocytes, Chap 7. In: In vitro lymphocyte transformation and antibody formation, 2nd edn., Year Book Medical Publishers, Chicago, pp 381–457

Keller JM, McClellan-Green PD, Lee AM, Arendt MD, Maier PP, Segars AL, Whitaker JD, Keil DE, Peden-Adams MM (2005) Mitogen-induced lymphocyte proliferation in logger head sea turtles: comparison of methods and effects of gender, plasma testosterone concentration and body condition on immunity. Vet Immunol Immunopathol 103:269–281

Nikitin PA, Price AM, McFadden K, Yan CM, Luftig MA (2014) Mitogen-induced B-cell proliferation activates Chk2-dependent G1/S cell cycle arrest. PLoS One 9:e87299

Sensi M, di Mario U, Pozzilli P (1984) Lymphocyte populations. Evaluation of T and B populations, T cell subpopulations and K cells. In: Larner J, Pohl SL (eds) Methods in diabetes research, vol I, Laboratory methods, Part B. Wiley, New York, pp 77–97

Yamamura M, Nikbin B, Hobbs JR (1976) Standardisation of the mixed lymphocyte reaction. J Immunol Methods 10:367–378

Zan-Bar I (1983) Modulation of B and T cell subsets in mice treated with fractionated total lymphoid irradiation. I. Blockade of differentiating B cell pathways. Eur J Immunol 13:35–40

Inhibition of T Cell Proliferation

Purpose and Rationale

Activation and/or proliferation of clonal populations of T cells are critical for the initiation of an antigen-specific immune response. Thus, inhibition of T cell activation provides a potent means for suppressing specific immune response. A number of immunosuppressive agents exhibit the ability to suppress T cell activation.

Procedure

Purification of Peripheral Blood Leukocytes and T Cells

Peripheral blood leukocytes from normal donors are separated on Ficoll-Hypaque (Pharmacia, Piscataway, NJ). Leukocyte suspensions are washed in HBSS and are resuspended in RPMI 1664 medium (Gibco, Grand Island, NY) containing 10 % heat-inactivated fetal bovine serum and 100 U/ml penicillin/streptomycin. Leukocyte suspensions are resuspended in RPMI 1664 containing 10 % heat-inactivated pooled human serum. Highly enriched T cells are obtained by passing leukocytes through a nylon wool column to remove macrophages and B cells and then depleted of NK and monocytes with anti-Leu 11 b (Becton Dickinson, Mountain View, CA) plus complement (Pel-Freez, Brown Deer, WI). These highly enriched T cells are approximately 95 % CD^{3+} cells, the remaining cells being B lymphocytes.

Mixed Lymphocyte Reaction

Peripheral blood leukocytes are incubated at 2×10^5/well with equal numbers of gamma-irradiated (3,000 rads) allogenic peripheral blood leukocytes and various concentrations of test compounds. Assays are performed in triplicate in 96-well, U-bottom plates. After 6 days of coculture, the cells are pulsed for 6 h with 1 μC of [^{3}H]thymidine per well. [^{3}H]Thymidine incorporation is then measured by scintillation counting. Data are presented as

$$\% \text{ inhibition} = \frac{\text{CPM}_{\text{expt}} - \text{CPM}_{\text{bckgrd}}}{\text{CPM}_{\text{ctrl}} - \text{CPM}_{\text{bckgrd}}} \times 100$$

where CPM_{expt} is mean counts per min of experimental cultures; $\text{CPM}_{\text{bckgrd}}$ is mean counts per min of background well, unstimulated cultures; and CPM_{ctrl} is mean counts per min of uninhibited, stimulated cultures.

Lymphocyte Stimulation and Proliferation

Peripheral blood leukocytes and isolated T cells are cultured with anti-CD3 (5 ng/ml) plus PMA (5 ng/ml), anti-CD28 (1:5,000 dilution) plus PMA (5 ng/ml), or 100 U/ml rhuIL-2 in RPMI 1644 containing 10 % fetal bovine serum. Peripheral blood leukocytes or T cells are cultured at 2×10^5 cell per well in a total volume of 200 µl/well. Assays are performed in quadruplicate in 96-well, U-bottom plates. [^{3}H]Thymidine (1 µC) is added to each well after 48 h of coculture, and after a 20 h pulse of [^{3}H] thymidine, the cells are harvested, and the amount of [^{3}H]thymidine uptake is quantitated on a scintillation counter.

ELISA Assays

Supernatants/well (100 ml) are harvested 24 h after initiation of cultures of peripheral blood leukocytes or T cells stimulated with anti-CD3 or anti-CD28 plus PMA. IL-2 in the coculture supernatant is quantitated using a commercially available IL-2 ELISA kit. All experiments are performed in duplicate.

IL-2R Assays

The expression of IL-2R on T cells stimulated for 48 h with anti-CD3 or anti-CD28 plus PMA is determined using FITC-conjugated anti-CD25 mABs (Becton Dickinson, Mountain View, CA). T cells are washed in HBSS and then stained with phycoerythrin-conjugated anti-CD3 mAB and fluorescein-conjugated antiCD25 mAB. The percent of cells coexpressing CD3+ and CD25+ is determined from 2,000 cells using an EPICS C flow cytometer (Coulter, Hialeah, FL).

Evaluation

Dose–response curves of inhibition of one-way mixed lymphocyte reaction and of IL-2 in the supernatant after stimulation with antiCD3 or anti-CD28 are established.

Modifications of the Method

Zielinski et al. (1993, 1994) studied the influence of leflunomide on expression of lymphocyte activation expression markers (IL-2 and transferrin receptors) as well as on cell cycle and on IL-2 receptor gene expression.

Calcineurin was found to be a key signaling enzyme in T lymphocyte activation and the target of immunosuppressive drugs (Clipstone and Crabtree 1993).

The viability and function of T lymphocytes has been explored using different cellular isolation techniques (Klein et al. 2006). A number of different vehicles have been shown to inhibit T cell proliferation which include the natural product silymarin (Morishima et al. 2010), heavy metals and polychlorinated biphenyls (Frouin et al. 2010), alternatively activated macrophages (Huber et al. 2010), type I interferon (Marshall et al. 2011), mesenchymal stem cells (Zinocker and Vaage 2012), and the programmed cell death-1 receptor (Patsoukis et al. 2015).

References and Further Reading

Chong ASF, Finnegan A, Jiang XL, Gebel H, Sankary HN, Foster P, Williams JW (1993a) Leflunomide, a novel immunosuppressive agent. Transplantation 55:1361–1366

Chong ASF, Gebel H, Finnegan A, Petraitis EE, Jiang XL, Sankary HN, Foster P, Williams JW (1993b) Leflunomide, a novel immunomodulatory agent: in vitro analyses of the mechanism of immuno-suppression. Transplant Proc 25:747–749

Clipstone NA, Crabtree GR (1993) Calcineurin is a key signaling enzyme in T lymphocyte activation and the target of the immunosuppressive drugs cyclosporin A and FK506. Ann N Y Acad Sci 696:20–30

Dayton JS, Turka LA, Thompson CB, Mitchell BS (1992) Comparison of the effects of mizoribine with those of azathioprine, 6-mercaptopurine, and mycophenolic acid on T lymphocyte proliferation and purine

ribonucleotide metabolism. Mol Pharmacol 41:671–676
di Padova FE (1989) Pharmacology of cyclosporine (Sandimmune) V. Pharmacological effects on immune function: in vitro studies. Pharmacol Rev 41:373–405
Frouin H, Menard L, Measures L, Brousseau P, Fournier M (2010) T lymphocyte-proliferative responses of a grey seal (*Halichoerus grypus*) exposed to heavy metals and PCBs in vitro. Aquat Mamm 36:365–371
Huber S, Hoffmann R, Muskens F, Voehringer D (2010) Alternatively activated macrophages inhibit T-cell proliferation by stat6-dependent expression of PD-L2. Blood 116:3311–3320
Klein AB, Witonsky SG, Ahmed SA, Holladay SD, Gogal RM Jr, Link L, Reilly CM (2006) Impact of different cell isolation techniques on lymphocyte viability and function. J Immunoassay Immunochem 27:61–76
Marshall HD, Urban SL, Welsh RM (2011) Virus-induced transient immune suppression and the inhibition of T cell proliferation by type I interferon. J Virol 85:5929–5939
Patsoukis N, Sari D, Boussiotis VA (2012) PD-1 inhibits T cell proliferation by upregulating p27 and p15 and suppressing cdc-25A. Cell Cycle 11:1–5
Morishima C, Shuhart MC, Wang CC, Paschal DM, Apodaca MC, Liu Y, Sloan DD, Graf TN, Oberlies NH, Lee DY-W, Jerome KR, Polyak SJ (2010) Silymarin inhibits in vitro T-cell proliferation and cytokine production in hepatitis C virus infection. Gastroenterology 138:671–681
Yamamura M, Nikbin B, Hobbs JR (1976) Standardisation of the mixed lymphocyte reaction. J Immunol Methods 10:367–378
Zielinski T, Müller HJ, Bartlett RR (1993) Effects of leflunomide (HWA 486) on expression of lymphocyte activation markers. Agents Actions 38(Spec Conf Issue):C80–C83
Zielinski T, Herrmann M, Müller HJ, Riedel N, Bartlett RR (1994) The influence of leflunomide on cell cycle, IL-2-receptor (IL-2-R) and its gene expression. Agents Actions 41(Spec Conf Issue):C204–C205
Zinocker S, Vaage JT (2012) Rat mesenchymal stromal cells inhibit T cell proliferation but not cytokine production through inducible nitric oxide synthase. Front Immunol 3:1–13

Chemiluminescence in Macrophages

Purpose and Rationale

The stimulation of macrophages by antigen, complement, phorbolesters, etc., leads to elaboration of O_2^- and other oxygen metabolites. Superoxide ion (O_2^-) and other highly reactive oxygen metabolites (radicals) form the basis for an efficient microbicidal system in vivo. Yet, when these radicals are released in response to self-antigens, tissue damage is often the result. Inhibition of this process can be regarded as a measure for immunomodulating effects of compounds. The oxygen metabolites can produce light-emitting reactions (chemiluminescence), which is measurable if amplified with suitable agents, such as the cyclic hydrazide luminol.

Procedure

NMRI mice weighing 30 g or Sprague–Dawley rats weighing 250–300 g of either sex are used.

Positive Control

1. Sensitized mice, receiving vehicle
2. Mice, developing an autoimmune disease, receiving vehicle
3. Rats, developing adjuvant arthritis, receiving vehicle

Negative Control

1. Mice not sensitized, receiving vehicle
2. Mice, not developing an autoimmune disease, receiving vehicle
3. Rats without adjuvant arthritis

Materials

- 5×10^8 SRBC (sheep red blood cells)/0.5 ml 0.9 % NaCl solution (for sensitization)
- *Phorbolester:* Stock solution of 1 mg/ml phorbolmyristenacetate. This stock solution is diluted with Hank's balanced salt solution to a final concentration of 3.5 μM (working solution). For the induction of chemiluminescence,

the working solution is diluted in the test tube 1:4, resulting in a final phorbolester concentration of 0.875 µM.
- Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma) final concentration 25 µg/ml

Ex Vivo Experiment

Groups of six animals are treated for 6 days orally or subcutaneously with test compound or the standard (prednisolone acetate or leflunomide). They are decapitated and exsanguinated. Macrophages are obtained by flushing the peritoneal cavity with 10 ml saline, containing 250 IU heparin. The cells are pooled, washed several times, and suspended again at a final concentration of 2×10^6/200 µl.

For measurement in the luminometer, the following mixture is prepared:

200 µl macrophages (2×10^6)
100 µl luminol solution (100 µg/ml)
100 µl phorbolmyristenacetate solution (3.5 µM)

Each sample is mixed thoroughly without the phorbolmyristenacetate solution, put into the luminometer, and counted at 2 min intervals for 10 s. The addition of the phorbolester induces the reaction.

In Vitro Experiment

To 100 µl of macrophage suspension (2×10^6 cells) is added 100 µl of the solution of the test compound and incubated for 15 min at 37 °C.

Then, 100 µl of luminol solution (100 µg/ml) and 100 µl of the 3.5 µM phorbolester solution are added and the luminescence measured in the luminometer.

Evaluation

The time of maximal counts for the positive control is recorded. For all groups, the ratio of counts per 10 s is determined at that time, compared to the positive control counts per 10 s, and the percent change is calculated. For statistical evaluation, the experimental group is compared with the positive control group using Student's *t*-test.

Modifications of the Method

Bird and Giroud (1985) described a technique of polymorphonuclear leukocyte chemiluminescence as a means to detect compounds with anti-inflammatory activity. Inflammatory polymorphonuclear leukocytes were obtained by injecting rats intrapleurally with 1 ml of a 1 % solution of calcium pyrophosphate and collection of the pleural exudate 4 h later. Chemiluminescence responses were measured using a Packard Picolite chemiluminometer and opsonized zymosan as the stimulus.

Seeds et al. (1985) found an independent stimulation of membrane potential changes and the oxidative metabolic burst in polymorphonuclear leukocytes.

A microtechnique for studying chemiluminescence response of phagocytes using whole blood was described by Selvaraj et al. (1982).

Traykov et al. (1997) investigated the effects of phenothiazine compounds on activated macrophage-induced luminal-dependent chemiluminescence, and Szliszka et al. (2013) studied the anti-inflammatory activity of artepillin C, a constituent of the resinous green propolis. Van Dyke et al. (2003) explored the use of lucigenin-based chemiluminescence assay to interrogate various inflammatory stages.

References and Further Reading

Bartlett RR (1986) Immunopharmacological profile of HWA 486, a novel isoxazol derivative-II. in vivo immunomodulating effects differ from those of cyclophosphamide, prednisolone, or cyclosporin A. Int J Immunopharmacol 8:199–204

Bird J, Giroud JP (1985) An appraisal of the technique of polymorphonuclear leukocyte chemiluminescence as a means to detect compounds with antiinflammatory activity. J Pharmacol Methods 14:305–312

Johnson RB Jr, Codzik CA, Cohn ZA (1978) Increased superoxide anion production by immunologically activated and chemically elicited macrophages. J Exp Med 148:115–120

Kurosawa M, Hanawa K, Kobayashi S, Nakano M (1990) Inhibitory effects of azelastine on superoxide anion generation from activated

inflammatory cells measured by a simple chemiluminescence method. Arzneim Forsch/ Drug Res 40:767–770
Merétey K, Boehm U, Falus A (1983) Chemiluminescence response of human blood mononuclear cells to PAH and histamine. Agents Actions 13:237–240
Seeds MC, Parce JW, Szeijda P, Bass DA (1985) Independent stimulation of membrane potential changes and the oxidative metabolic burst in polymorphonuclear leukocytes. Blood 65:233–240
Selvaraj R, Sbarra AJ, Thomas GB, Cetrulo CL, Mitchell GW (1982) A microtechnique for studying chemiluminescence response of phagocytes using whole blood and its application to the evaluation of phagocytes in pregnancy. J Reticuloendothel Soc 31:3–16
Szliszka E, Mertas A, Czuba ZP, Krol W (2013) Inhibition of inflammatory response by Artepillin C in activated RAW264.7 macrophages. Evid Based Complement Alternat Med. doi:10.1155/2013/735176
Traykov T, Hadjimitova V, Golivsky P, Ribarov S (1997) Effect of phenothiazines on activated macrophage-induced luminal-dependent chemiluminescence. Gen Physiol Biophys 16:3–14
Van Dyke K, Patel S, Vallyathan V (2003) Lucigenin chemiluminescence assay as an adjunctive tool for assessment of various stages of inflammation: a study of quiescent inflammatory cells. J Biosci 28:115–119
Weidemann MJ, Smith R, Heaney T, Alaudeen S (1980) On the mechanism of the generation of chemiluminescence by macrophages. Behring Inst Mitt 65:42–54
Weinberg JB, Misokonis MA (1983) Phorbol diester-induced H_2O_2 production by peritoneal macrophages. Cell Immunol 80:405–415

PFC (Plaque-Forming Colony) Test In Vitro

Purpose and Rationale

Identification of antibody-producing cells is based on the ability of the secreted IgM antibody to fix complement and thereby lyse the indicator erythrocytes. Spleen cells or peripheral blood lymphocytes, previously incubated with antigen, are mixed with sheep red blood cells (SRBC). After addition of complement and incubation, plaques (clear areas) caused by the lysis of SRBC appear in the otherwise cloudy layer. Antibody-forming cells can be detected by the appearance of plaques. The number of plaques obtained is proportional to the number of antibody-producing lymphocytes in the cell population.

Procedure

NMRI mice weighing 16–18 g or Lewis rats weighing 180–200 g of either sex are used.

Materials

- Absorbed guinea pig complement
- SRBC stored in Alsever's solution

Positive Control

Spleen cells incubated with antigen and medium

Negative Control

Spleen cells incubated with medium alone. The animals are decapitated and the spleens are removed from the peritoneal cavity. A single cell suspension of 15×10^6 cells/ml is prepared. For the induction of PFC, a 0.5 ml splenocyte suspension is added to 0.5 ml of a suspension of SRBC, previously washed in medium and diluted to 8×10^6 cells/ml. Thereafter, 1 ml of the solution of the test compound is added, and the limbrowells are incubated at 37 °C in a CO_2 incubator for 5 days. Per group 3 limbrowells are set up. On day 5, the three wells of each group are pooled and washed in medium, and the number of cells is determined. For each cell pellet, 875 µl of washed SRBC and 125 µl absorbed guinea pig complement are added. The suspension is mixed thoroughly and filled in chambers constructed of microslides. The chambers are placed in the incubator at 37 °C for 90–120 min. The plaque-forming colonies are counted immediately after incubation.

Evaluation

The activity of test compounds can be determined using the following formula:

1. PFC/3 wells:

$$x = \frac{\text{plaques} \times 100}{\mu\text{l}}$$

2. % change in the number of plaques:

$$x = \frac{\text{plaques} \times 100}{\text{plaquespos. control}}$$

$$d\% = x - 100$$

3. % change in number of cells:

$$x = \frac{\text{number of cells} \times 100}{\text{number of cells pos. control}}$$

$$d\% = x - 100$$

References and Further Reading

Bartlett RR (1986) Immunopharmacological profile of HWA 486, a novel isoxazol derivative – II. In vivo immunomodulating effects differ from those of cyclophosphamide, prednisolone, or cyclosporin A. Int J Immunopharmacol 8:199–204

Borel JF, Feurer C, Gubler HU, Stähelin H (1976) Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 6:468–475

Cunningham AJ, Szenberg A (1968) Further improvements in the plaque technique for detecting single antibody forming cells. Immunology 14:599–608

Stockinger (1978) Negative Rückkoppelungsmechanismen des Immunsystems. Johannes Gutenberg Universität Mainz, Mainz, Germany

Zaalberg OB (1964) A simple method for detecting single antibody-forming cells. Nature 202:1231

Inhibition of Dihydroorotate Dehydrogenase

Purpose and Rationale

Dihydroorotate dehydrogenase catalyzes the fourth committed step in the de novo biosynthesis of pyrimidines. As rapidly proliferating human T cells have an exceptional requirement for de novo pyrimidine biosynthesis, small-molecule dihydroorotate dehydrogenase inhibitors constitute an attractive therapeutic approach to autoimmune diseases, immunosuppression, and cancer. The main mode of action of the immunosuppressive compound leflunomide and its active metabolites is considered to be the inhibition of the enzyme dihydroorotate dehydrogenase (Bruneau et al. 1998; Graul and Castañer 1998; Knecht and Löffler 1998; Rückemann et al. 1998; Schorlemmer et al. 1998; Herrmann et al. 2000; Liu et al. 2000).

Procedure

A fragment of human dihydroorotate dehydrogenase is expressed by means of the baculovirus expression vector system and purified to a specific activity greater than 50 U/mg (Knecht et al. 1996, 1997). Enzyme assays are performed with purified recombinant dihydroorotate dehydrogenase at 30 °C. The oxidation of the substrate dihydroorotate and the reduction of the co-substrate quinone is coupled to the reduction of the chromogen 2,6-dichlorophenolindophenol (DCIP). The reaction mixture contains 0.1 mM Q_D or 0.1 M Q_{10}, 1 mM L-dihydroorotate, 0.06 mM DCIP, 0.1 % Triton X-100 in 50 mM Tris–HCl buffer, 150 mM KCl, and pH 8.0. The reaction is started by addition of the enzyme. The loss of absorbance of the blue DCIP is monitored at 600 nm: $\in = 18.800\ \text{l mol}^{-1}\ \text{cm}^{-1}$. The enzyme activity in control assays without Q_D or Q_{10} which is approximately 1 % of maximum enzyme activity is subtracted from the activity values measured. Stock solutions of the test compounds are prepared in dimethyl sulfoxide with further dilutions in the buffer taken for the assays.

Evaluation

To determine the inhibitory potency of the agents, the initial velocity of dihydroorotate dehydrogenase reaction is measured at saturating substrate concentrations, 1 mM dihydroorotate and 100 μM Q_D, and varying concentrations of the drugs (1 nM through 100 μM). The equation is fitted to the initial velocities:

$$v = V/\{1 + [I]/IC_{50}\}$$

([I] is the inhibitor concentration) in order to find the concentration causing 50 % inhibition of the enzyme activity (IC_{50}). Both virtual (Diao et al. 2012) and high-throughput screening (Baldwin et al. 2005) and have been used to identify micromolar and sub-micromolar, respectively, inhibitors of DHODH activity. Recently, DHODH has emerged as a therapeutic target in bovine babesiosis (2014).

References and Further Reading

Baldwin J, Michnoff CH, Malmquist NA, White J, Roth MG, Rathod PK, Phillips MA (2005) High-throughput screening for potent and selective inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase. J Biol Chem 280:21847–21853

Bruneau JM, Yea CM, Spinella-Jaegle S, Fudali C, Woodward K, Robson PA, Sautès C, Westwood R, Kuo EA, Williamson RA, Ruuth E (1998) Purification of human dihydro-orotate dehydrogenase and its inhibition by A77 1726, the active metabolite of leflunomide. Biochem J 336:299–303

Diao Y, Lu W, Huangtao J, Zhu J, Han L, Xu M, Gao R, Shen X, Zhao Z, Liu X, Xu Y, Huang J, Li H (2012) Discovery of diverse human dihydroorotate dehydrogenase inhibitors as immunosuppressive agents by structure-based virtual screening. J Med Chem 55:8341–8349

Graul A, Castañer J (1998) Leflunomide. Drugs Future 23:827–837

Herrmann ML, Schleyerbach R, Kirschbaum BJ (2000) Leflunomide: an immunomodulatory drug for the treatment of rheumatoid arthritis and other autoimmune diseases. Immunopharmacology 47:273–289

Kamyingkird K, Cao S, Masatani T, Moumont PFA, Vudriko P, Mousa AAEM, Terkawi MA, Nishikawa Y, Igarashi I, Xuan X (2013) Babesia bovis dihydroorotate dehydrogenase (BboDHODH) is a novel molecular target of drug for bovine babeosis. J Vet Med Sci 76:323–330

Knecht W, Löffler M (1998) Species-related inhibition of human and rat dihydroorotate dehydrogenase by immunosuppressive isoxazol and cinchoninic acid derivatives. Biochem Pharmacol 56:1259–1264

Knecht W, Bergjohann U, Gonski S, Kirschbaum B, Löffler M (1996) Functional expression of a fragment of human dihydroorotate dehydrogenase by means of the baculovirus vector system, and kinetic investigation of the purified enzyme. Eur J Biochem 240:292–301

Knecht W, Altekruse D, Rotgeri A, Gonski S, Löffler M (1997) Rat dihydroorotate dehydrogenase: isolation of the recombinant enzyme from mitochondria of insect cells. Protein Expr Purif 10:89–99

Liu S, Neidhardt EA, Grossman TH, Ocain T, Clardy J (2000) Structures of human dihydroorotate dehydrogenase in complex with antiproliferative agents. Struct Fold Des 8:25–33

Lukens AK, Ross LS, Heidebrecht R, Gamo FJ, Lafuente-Monasterio MJ, Booker ML, Hartl DL, Wiegand RC (2014) Wirth DF Harnessing evolutionary fitness in *Plasmodium falciparum* for drug discovery and suppressing resistance. Proc Natl Acad Sci USA 111: 799–804

Rückemann K, Fairbanks LD, Carrey EA, Hawrylowicz CM, Richards DF, Kirschbaum B, Simmonds HA (1998) Leflunomide inhibits pyrimidine de novo synthesis in mitogen-stimulated T-lymphocytes from healthy humans. J Biol Chem 273:21682–21691

Schorlemmer HU, Milbert U, Haun G, Wunschel M, Zeitter D, Schleyerbach R

(1998) De novo pyrimidine biosynthesis in Jurkat T cells is inhibited by leflunomide's primary metabolite A77–1726 at the level of dihydroorotate dehydrogenase. Int J Immunother 14:193–204

Sphingosine 1-Phosphate

General Considerations

Sphingolipids have emerged as molecules whose metabolism is regulated to generation of bioactive products including ceramide, sphingosine, and sphingosine-1-phosphate. The balance between cellular levels of these bioactive products is recognized to be critical to cell regulation and may be a promising approach to tumor therapy and multiple sclerosis (Huwiler and Pfeilschifter 2006; Rosen et al. 2013; Blaho and Hla 2014), whereby ceramide and sphingosine cause apoptosis and growth arrest phenotypes and sphingosine-1-phosphate mediates proliferative and angiogenic responses. Sphingosine kinase is a key enzyme in modulating the levels of these lipids (Hannun and Obeid 1995; Hofmann and Dixit 1998; Mathias et al. 1998; Prieschl et al. 1999; Pyne and Pyne 2000; Cummings et al. 2002; MacKinnon et al. 2002; Rosen and Liao 2003; Chen et al. 2004; Deguchi et al. 2004; Lee et al. 2004; Peng et al. 2004; Cyster 2005; Kee et al. 2005; Watterson et al. 2005; Gardell et al. 2006; Taha et al. 2006). Ceramide formation and degradation are influenced by nitric oxide (NO) (Huwiler et al. 1999a, b; Franzen et al. 2002a, b).

References and Further Reading

Blaho VA, Hla T (2014) An update on the biology of sphingosine-1-phosphate receptors. J Lipid Res. doi:10.1194/jlr.R046300

Chen XL, Grey JY, Thomas S, Qiu FH, Medford RM, Wasserman MA, Kunsch C (2004) Sphingosine kinase-1 mediates TNF-α induced MCP-1 gene expression in endothelial cells: upregulation by oscillatory flow. Am J Physiol 287:H1452–H1458

Chun J, Rosen H (2006) Lysophospholipid receptors as potential drug targets in tissue transplantation and autoimmune diseases. Curr Pharm Des 12:161–171

Cummings RJ, Parinandi NL, Zaiman A, Wang L, Usatyuk PV, Garcia JGN, Natarajan V (2002) Phospholipase D activation by sphingosine 1-phosphate regulates interleukin-8 secretion in human bronchial epithelial cells. J Biol Chem 277:30227–30235

Cyster JG (2005) Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu Rev Immunol 23:127–159

Deguchi H, Yegneswaran S, Griffin JH (2004) Sphingolipids as bioactive regulators of thrombin generation. J Biol Chem 279: 12036–12042

Franzen R, Fabbro D, Aschrafi A, Pfeilschifter J, Huwiler A (2002a) Nitric oxide induces degradation of the neutral ceramidase in rat mesangial cells and is counterregulated by protein kinase C. J Biol Chem 277:46184–46190

Franzen R, Pfeilschifter J, Huwiler A (2002b) Nitric oxide induces neutral ceramidase degradation by the ubiquitin/proteasome complex in renal mesangial cell cultures. FEBS Lett 552:441–444

Gardell SE, Dubin AE, Chun J (2006) Emerging medical roles for lysophospholipid signaling. Trends Mol Med 12:65–75

Hannun YA, Obeid LM (1995) Ceramide: an intracellular signal for apoptosis. Trends Biol Sci 20:73–77

Hofmann K, Dixit VM (1998) Ceramide in apoptosis– does it really matter? Trends Biol Sci 23:374–377

Huwiler A, Pfeilschifter J, van den Bosch (1999a) Nitric oxide donors induce stress signaling via ceramide formation in rat renal mesangial cells. J Biol Chem 274:7190–7195

Huwiler A, Dorsch S, Briner V, van den Bosch H, Pfeilschifter J (1999b) Nitric oxide stimulates ceramide formation in glomerular endothelial cells. Biochem Biophys Res Commun 258:60–65

Huwiler A, Pfeilschifter J (2006) Altering the sphingosine-1-phosphate/ceramide balance: a

promising approach to tumor therapy. Curr Pharm Des 12:4625–4635
Kee TH, Vit P, Melendez AJ (2005) Sphingosine kinase signaling in immune cells. Clin Exp Pharmacol Physiol 32:153–161
Lee H, Lin CI, Liao JJ, Lee YW, Yang HY, Lee CY, Hsu HY, Wu HL (2004) Lysophospholipids increase ICAM-1 expression in HUVEC through a G_i- und NF-κB-dependent mechanism. Am J Physiol 287:C1657–C1666
MacKinnon AC, Buckley A, Chilvers ER, Rossi AG, Haslett C, Sethi T (2002) Sphingosine kinase: a point of convergence in the action of diverse neutrophil priming agents. J Immunol 169:6394–6400
Mathias S, Pena LA, Kolesnick RN (1998) Signal transduction of stress via ceramide. Biochem J 335:465–480
Peng X, Hassoun PM, Sammani S, McVerry BJ, Burne MJ, Rabb H, Pearse D, Tuder RM, Garcia JGN (2004) Protective effects of sphingosine 1-phsophate in murine endotoxin-induced inflammatory lung injury. Am J Respir Crit Care Med 169:1245–1251
Prieschl EE, Csonga R, Novotny V, Kikuchi GE, Baumruker T (1999) The balance between sphingosine and sphingosine-1-phosphate is decisive for mast cell activation after Fcε receptor I triggering. J Exp Med 190:1–8
Pyne S, Pyne NJ (2000) Sphingosine 1-phosphate signaling in mammalian cells. Biochem J 349:385–402
Rosen H, Liao J (2003) Sphingosine 1-phosphate pathway therapeutics: a lipid ligand-receptor paradigm. Curr Opin Chem Biol 7:461–46
Rosen H, Stevens RC, Hanson M, Roberts E, Oldstone MB (2013) Sphingosine-1-phosphate and its receptors: structure, signaling, and influence. Annu Rev Biochem 82:637–662
Taha TA, Hannun YA, Obeid LM (2006) Sphingosine kinase: biochemical and cellular regulation and role in disease. J Biochem Mol Biol 39:113–131
Watterson KR, Ratz PH, Spiegel S (2005) The role of sphingosine-1-phosphate in smooth muscle contraction. Cell Signal 17:289–298

Binding to Sphingosine 1-Phosphate Receptors

Purpose and Rationale

At least five subtypes of the sphingosine 1-phosphate receptor with tissue specificity are known (Meyer zu Heringdorf et al. 1998; Kon et al. 1999; Im et al. 2000, 2001; Forrest et al. 2004; Hale et al. 2004a; Sanna et al. 2004; Zhou and Murthy 2004; Xin et al. 2004; Lepley et al. 2005; Kimura et al. 2006; Kitano et al. 2006).

The immunomodulator FTY720 is an agonist to sphingosine 1-phosphate receptors (Brinkmann et al. 2002, 2010; Brunkhorst et al. 2014; Chiba 2005; Chiba et al. 2011, 2014; Gräler and Goetzl 2004; Kunzendorf et al. 2004; Xin et al. 2004; Albert et al. 2005; Bandhuvula et al. 2005; Sawicka et al. 2003, 2005; Habicht et al. 2005; Takasugi et al. 2013; Xin et al. 2006; Zhang et al. 2013; Zhou et al. 2006). FTY720 is derived from ISP-1 (myriocin), a fungal metabolite that is an eternal youth nostrum in traditional Chinese herbal medicine (Fujita et al. 1994). The compound {2-amino-2-[2-(4-octophenyl) ethyl]propane-1,3-diol} is a highly potent immune modulating agent.

Further derivates such as sphingosine 1-phosphate receptor agonists (Hale et al. 2004b, c; Clemens et al. 2005; Foss et al. 2005; Galicia-Rosas et al. 2012; Guerrero et al. 2013; Kiuchi et al. 2005; Komiya et al. 2012; Jin et al. 2014; Jo et al. 2005; Li et al. 2005; Colandrea et al. 2006; Sanada et al. 2011; Satsu et al. 2013; Sobel et al. 2013; Ren et al. 2012; Yamamoto et al. 2014) and antagonists (Davis et al. 2005; Kennedy et al. 2011; Angst et al. 2012) have been described, and a patent review of sphingosine 1-phosphate receptors has been conducted (Roberts et al. 2013). Brinkmann et al. (2002) used the [γ-^{35}S] GTPS-binding assay to study the binding of the immune modulator FTY720 to sphingosine 1-phosphate receptors.

Forrest et al. (2004) studied the binding of sphingosine 1-phosphate agonists on distinct receptor subtypes.

Procedure

Receptors and Cell Lines

CHO cells stably expressing human $S1P_{1,2,3,4,5}$ were used (Mandala et al. 2002). cDNA sequences encoding rodent S1P receptors were cloned from genomic DNA by polymerase chain reaction using the following primers for each respective receptor:

5′-GAACCCGGGTGTCCACTAGCATCCCGG and 5′CCCGAATTCTTAGGAAGAAGAATTGACGTTTCC (mouse $S1P_1$), 5′-GAACCCGGGCGGCTTATACTCAGAGTACC and 5′-GGCGAATTCTCAGACCACTGTGTTACCCTC (mouse $S1P_2$), 5′-GAACCCGGGCAACCACGCATGCGCAGG and 5′-GTCGAATTCTCACTTGCAGAGGACCCCG (mouse $S1P_3$), 5′-GAACCCGGGAACATCAGTACCTGGTCCACGC and GCGGAATTCTAGGTGCTGCGGACGCTGG (mouse $S1P_4$), 5′-GAACCCGGGCTGCTGCGGCCGG and 5′-CGCGAATTCAGTCTGTAGCAGTAGGCACC (mouse $S1P_5$), 5′-GTAGGATCCGTGTCCTCCACCAGCATC and 5′GGCCGAATTCTTAAGAAGAAGAATTGACGTTTC (rat $S1P_1$), 5′-GAACCCGGGCATCCACGCATGCGCAG and 5′-GCCGAATTCTCACTTGCAGAGGACCCCATTCTG (rat $S1P_3$).

The polymerase chain reaction products were inserted in-frame after a FLAG tag using vector pCMV-Tag2 (Stratagene, La Jolla, Calif., USA). Stable lines were established by transfecting plasmids into CHO cells using Lipofectamine reagent, selecting for neomycin resistance, and screening single cell cultures for increased [^{33}P] S1P-specific binding. Membranes were prepared from positive clones and confirmed in [^{33}P]S1P and [^{35}S]GTPγS binding assays.

S1P Receptor Assays

Binding assays were conducted as described by Mandala et al. (2002). [^{33}P]S1P was sonicated with fatty-acid-free bovine serum albumin, added to test compounds diluted in dimethyl sulfoxide (DMSO), and mixed with membranes in 200 µl in 96-well plates with assay concentrations of 0.1 nM[^{33}P]S1P (22,000 dpm), 0.5 % bovine serum albumin, 50 mM HEPES-Na (pH 7.5), 5 mM $MgCl_2$, 1 mM $CaCl_2$, and 0.3–0.7 µg of membrane protein. Binding was performed for 60 min at room temperature and terminated by collecting the membranes onto GF/B filter plates with a Packard Filtermate Universal harvester. Filter-bound radionuclide was measured on a Perkin Elmer 1450 MicroBeta. Specific binding was calculated by subtracting radioactivity that remained in the presence of 1,000-fold excess of unlabeled S1P.

To measure functional activation of the S1P receptors, [^{35}S]GTPγ *S* binding was measured. Membranes (1–4 µg of protein) were incubated in 96-well plates with test compounds diluted in DMSO in 100 µl of buffer containing 20 mM HEPES (pH 7.4), 100 mM NaCl, 10 mM $MgCl_2$, and 2–10 µM GDP, depending on the expressed receptor. The assay was initiated with the addition of 100 µl of [^{35}S]GTPγ S (1,200 Ci/mmol or 44,400 BGq/mmol; Perkin Elmer Life and Analytical Sciences, Boston, Mass., USA) for an assay concentration of 125 pM. After 60 min of incubation at room temperature, membranes were harvested onto GF/B filter plates, and bound radionuclides were measured.

Modifications of the Method

Murata et al. (2000) described a radioreceptor-binding assay for quantitative measurement of sphingosine 1-phosphate.

References and Further Reading

Albert R, Hinterding K, Brinkmann V, Guerini D, Müller-Hartwieg C, Knecht H, Simeon C, Streiff M, Wagner T, Welzenbach K, Zecri F, Zollinger M, Cooke N, Francotte E (2005) The novel immunomodulator FTY720 is phosphorylated in rats and humans to form a single stereoisomer. Identification, chemical proof, and biological characterization of the biologically active species and its enantiomer. J Med Chem 48:5373–5377

Angst D, Janser P, Quancard J, Buehlmayer P, Berst F, Oberer L, Beerli C, Streiff M, Pally C, Hersperger R, Bruns C, Bassilana F, Bollbuck B (2012) An oral sphingosine

1-phosphate receptor 1 ($S1P_1$) antagonist prodrug with efficacy in vivo: discovery, synthesis, and evaluation. J Med Chem 55:9722–9734

Bandhuvula P, Tam YY, Oskoulan B, Saba JD (2005) The immune modulator FTY720 inhibits sphingosine-1-phosphate lyase activity. J Biol Chem 280:33697–33700

Bolli MH, Lescop C, Nayler O (2011) Synthetic sphingosine 1-phosphate receptor modulators – opportunities and potential pitfalls. Curr Top Med Chem 11:726–757

Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457

Brinkmann V, Billich A, Baumraker T, Heining P, Schmouder R, Rancis G, Aradhye S, Burtin P (2010) Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. Nat Rev Drug Discov 9:883–897

Brunkhorst R, Vutukuri R, Pfeilschifter W (2014) Fingolimod for the treatment of neurological diseases-state of play and future perspectives. Front Cell Neurosci 8:283 doi:10.3389/fncel.2014.00283

Chiba K (2005) FTY720, a new class of immunomodulators, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther 108:308–319

Chiba C, Kataoka H, Seki N, Maeda Y, Sugahara K (2011) Fingolimod (FTY720), the sphingosine 1-phosphate receptor modulator, as a new therapeutic drug in multiple sclerosis. Inflamm Regen 31:167–174

Clemens JJ, Davis MD, Lynch KR, Macdonald TL (2005) Synthesis of 4(5)-phenylimidazole-based analogues of sphingosine 1-phosphate and FTY720: discovery of potent $S1P_1$ receptor agonists. Bioorg Med Chem Lett 15:3568–3572

Colandrea VJ, Legiec IE, Huo P, Yan L, Hale JJ, Mills SG, Bergstrom J, Card D, Chebret G, Hajdu R, Keohane CA, Milligan JA, Rosenbach MJ, Shei GJ, Mandala SM (2006) 2,5-Disubstituted pyrrolidines carboxylates as potent, orally active sphingosine-1-phosphate (S1P) receptor agonists. Bioorg Med Chem Lett 16:2905–2908

Davis MD, Clemens JJ, Macdonald TL, Lynch KR (2005) Sphingosine 1-phosphate analogs as receptor antagonists. J Biol Chem 280:9833–9844

Forrest M, Sun SY, Hajdu R, Bergstrom J, Card D, Doherty G, Hale J, Keohane C, Meyers C, Milligan J, Mills S, Nomura H, Rosen H, Rosenbach M, Shei GJ, Singer II, Tian M, West S, White V, Xie J, Proia RL, Mandala S (2004) Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate agonists in rodents are mediated via distinct receptor subtypes. J Pharmacol Exp Ther 309:758–768

Foss FW Jr, Clemens JJ, Davis MD, Snyder AH, Zigler MA, Lynch KR, Macdonald TL (2005) Synthesis, stability, and implications of phosphothioate agonists of sphingosine-1-phosphate receptors. Bioorg Med Chem Lett 15:4470–4474

Fujita T, Inoue K, Yamamoto S, Ikumoto T, Sasaki S, Toyama R, Chiba K, Hoshima Y, Okumato T (1994) Fungal metabolites. Part II. A potent immunosuppressive activity found in *Isaria sinclairii* metabolite. J Antibiot 47:208–215

Galicia-Rosas G, Pikor N, Schwartz JA, Rojas O, Jian A, Summers-Deluca L, Ostrrowski M, Nuesslein-Hildesheim B, Gommerman JL (2012) A sphingosine-1-phosphate receptor-1 directed agonist reduces central nervous system inflammation in a plasmacytoid dendritic cell-dependent manner. J Immunol 189:3700–3706

Gräler MH, Goetzl EJ (2004) The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. FASEB J 18:551–553

Guerrero M, Poddutoori R, Urbano M, Peng X, Spicer TP, Chase PS, Hodder PS, Schaeffer M-T, Brown S, Rosen H, Roberts E (2013)

Discovery, design and synthesis of a selective S1P3 receptor allosteric agonist. Bioorg Med Chem 23:6346–6349

Habicht A, Clarkson MR, Yang J, Henderson J, Brinkmann V, Fernandes S, Jurewicz M, Yuan X, Sayegh MH (2005) Novel insights into the mechanism of action of FTY720 in a transgenic model of allograft rejection: implications for therapy of chronic rejection. J Immunol 176:36–42

Hale JJ, Doherty G, Toth L, Li Z, Mills SG, Hajdu R, Keohane CA, Rosenbach M, Milligan J, Shei GJ, Chrebet G, Bergstrom J, Card D, Rosen H, Mandala S (2004a) The discovery of 3-(*N*-alkyl) aminopropylphosphonic acids as potent S1P receptor agonists. Bioorg Med Chem Lett 14:3495–3499

Hale JJ, Yan L, Neway WE, Hajdu R, Bergstrom JD, Milligan JA, Shei GJ, Chrebet GL, Thornton RA, Card D, Rosenbach E, Rosen H, Mandala S (2004b) Synthesis, stereochemical determination and biochemical characterization of the enantiomeric phosphate esters of the novel immunosuppressive agent FTY720. Bioorg Med Chem 12:4803–4807

Hale JJ, Neway W, Mills SG, Hajdu R, Keohane CA, Rosenbach M, Milligan J, Shei GJ, Chrebet G, Bergstrom J, Card D, Koo GC, Koprak SL, Jackson JJ, Rosen H, Mandala S (2004c) Potent S1P receptor agonists replicate the pharmacologic actions of the novel immune modulator FTY720. Bioorg Med Chem Lett 14:3351–3355

Im DS, Heise CE, Ancellin N, O'Dowd BF, Shei GJ, Heavens RP, Rigby MR, Hla T, Mandala S, McAllister G, George SR, Lynch KR (2000) Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. J Biol Chem 275:14281–14286

Im DS, Clemens J, Macdonald T, Lynch KR (2001) Characterization of the human and mouse sphingosine 1-phosphate receptor, $S1P_5$ (Edg-8): structure-activity relationship of sphingosine 1-phosphate receptors. Biochemistry 40:14053–14060

Jin J, Hu J, Zhou W, Wang X, Xiao Q, Xue N, Yin D, Chen X (2014) Development of a selective $S1P_1$ receptor agonist, Syl930, as a potential therapeutic agent for autoimmune encephalitis. Biochem Pharmacol 90:50–61

Jo E, Sanna MG, Gonzalez-Cabrera PJ, Thangada S, Tigyl G, Osborne DA, Hla T, Parill ASL, Rosen H (2005) $S1P_1$-selective in vivo-active agonists from high-throughput screening: off-the-shelf chemical probes of receptor interactions, signaling and fate. Chem Biol 12:703–715

Kennedy PC, Zhu R, Huang T, Tomsig JL, Mathews TP, David M, Peyruchard OO, Macdonald TL, Lynch KR (2011) Characterisation of a sphingosine 1-phosphate receptor antagonist prodrug. J Pharmacol Exp Ther 338:879–889

Kimura T, Tomura H, Mogi C, Kuwabara A, Ishiwara M, Shibasawa K, Sato K, Ohwada S, Im DS, Kurose H, Ishizuka T, Murakami M, Okajima F (2006) Sphingosine 1-phosphate receptors mediate stimulatory and inhibitory signalings for expression of adhesion molecules in endothelial cells. Cell Signal 18:841–850

Kitano M, Hla T, Sekiguchi M, Kawahito Y, Yoshimura R, Miyazawa K, Iwasaki T, Sano H (2006) Sphingosine 1-phosphate/sphingosine 1-phosphate receptor 1 signaling in rheumatoid synovium. Regulation of synovial proliferation and inflammatory gene expression. Arthritis Rheum 54:742–753

Kiuchi M, Adachi K, Tomatsu A, Chino M, Takeda S, Tanaka Y, Maeda Y, Sato N, Mitsutomi N, Sugahara K, Chiba K (2005) Asymmetric synthesis and biological evaluation of the enantiomeric isomers of the immunosuppressive FTY720-phosphate. Bioorg Med Chem 13:425–432

Komiya T, Sato K, Shioya H, Inagaki Y, Hagiya H, Kozaki R, Imai M, Takada Y, Maeda T, Kurata H, Kurono M, Suzuki R, Otsuki K, Habashita H, Nakade S (2012) Efficacy and immunomodulatory actions of ONO-4641, a novel selective agonist for sphingosine 1-phosphate receptors 1 and 5, in pre-clinical models of multiple sclerosis. Clin Exp Immunol 171:454–62

Kon J, Sato K, Watanabe T, Tomura H, Kuwabara A, Kimura T, Taman KI, Ishizuka T, Murata Nkanda T, Kobayashi I, Ohta H, Ui M, Okajima F (1999) Comparison of intrinsic activities of the putative sphingosine 1-phosphate receptor subtypes to regulate several signaling pathways in their transfected Chinese hamster ovary cells. J Biol Chem 274:23940–23947

Kunzendorf U, Ziegler E, Kabelitz D (2004) FTY720-the first compound of a new promising class of immunosuppressive drugs. Nephrol Dial Transplant 19:1677–1681

Lepley D, Paik JH, Hla T, Ferrer F (2005) The G protein-coupled receptor $S1P_2$ regulates Rho/Rho kinase pathway to inhibit tumor cell migration. Cancer Res 65:3788–3795

Li Z, Chen W, Hale JJ, Lynch CL, Mills SG, Hajdu R, Keohans CA, Rosenbach MJ, Milligan JA, Shei GJ, Chrebet G, Parent SA, Bergstrom J, Card D, Forrest M, Quackenbush EJ, Wickham LA, Vargas H, Evans TRM, Rosen H, Mandala S (2005) Discovery of potent 3,5-diphenyl-1,2,4-oxadiazole sphingosine 1-phosphate ($S1P_1$) receptor agonists with exceptional selectivity against $S1P_2$ and $S1P_3$. J Med Chem 48:6169–6173

Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thronton R, Shei GJ, Card D, Keohane CA, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2002) Alteration of lymphocyte trafficking by sphingosine receptor agonists. Science 296:346–349

Meyer zu Heringdorf D, Lass H, Alemany R, Laser KT, Neumann E, Zhang C, Schmidt M, Rauen U, Jakobs KH, van Koppen CJ (1998) Sphingosine kinase-mediated Ca2+ signalling by G-protein-coupled receptors. EMBO J 17:2830–2837

Murata N, Sato K, Kon J, Tomura H, Okajima F (2000) Quantitative measurement of sphingosine 1-phosphate by radioreceptor-binding assay. Anal Biochem 282:115–120

Ren F, Deng G, Wang H, Luan L, Meng Q, Xu Q, Xu H, Xu X, Zhang H, Zhao B, Li C, Guo TB, Yang J, Zhang W, Zhao Y, Jia Q, Lu H, Xiang J-N, Elliott JD, Lin X (2012) Discovery of novel 1,2,4-thiadiazole derivatives as potent, orally active agonists of sphingosine 1-phosphate receptor subtype 1 ($S1P_1$). J Med Chem 55:4286–4296

Roberts E, Guerrero M, Urbano M, Rosen H (2013) Sphingosine 1-phosphate receptor agonists: a patent review (2010–2012). Expert Opin Ther Pat 23:817–841

Sanada Y, Mizushima T, Kai Y, Nishimura J, Hagiya H, Kurata H, Mizuno H, Uejima E, Ito T (2011) Therapeutic effects of novel sphingosine-1-phosphate receptor agonist W-061 in murine DSS colitis. PLoS One 6: e23933

Sanna MG, Liao J, Jo E, Alfonso C, Ahn MY, Peterson MS, Webb B, Lefebvre S, Chun J, Gray N, Rosen H (2004) Sphingosine 1-phosphate (S1P) receptor subtypes $S1P_1$ and $S1P_3$, respectively, regulate lymphocyte recirculation and heart rate. J Biol Chem 279:13839–13848

Satsu H, Schaeffer M-T, Guerro M, Saldana A, Eberhardt C, Hodder P, Cayanan S, Bhhatarai B, Roberts E, Rosen H, Brown SJ (2013) A sphingosine 1-phosphate receptor 2 selective allosteric agonist. Bioorg Med Chem 21:5373–5382

Sawicka E, Zuany-Amorim C, Manlius C, Trifilieff A, Brinkmann V, Kemeny DM, Walker C (2003) Inhibition of Th1- and Th2-mediated airway inflammation by the sphingosine 1-phosphate receptor agonist FTY720. J Immunol 171:6206–6214

Sawicka E, Dubois G, Jarai G, Edwards M, Thomas M, Nicholls A, Albert R, Newson C, Brinkmann V, Walker C (2005) The sphingosine 1-phosphate receptor agonist FTY720 differentially affects the sequestration of $CD4^+/CD25^+$ T-regulatory cells and enhances their functional activity. J Immunol 175:7973–7980

Sobel K, Menyhart K, Killer N, Renault B, Bauer Y, Studer R, Steiner B, Bolli MH, Nayler O, Gatfield J (2013) Sphingosine 1-phosphate (S1P) receptor agonists mediate

pro-fibrotic responses in normal human lung fibroblasts via $S1P_2$ and $S1P_3$ receptors and Smad-independent signaling. J Biol Chem 288:14839–14851

Takasugi N, Sasaki T, Ebinuma I, Osawa S, Isshiki H, Takeo K, Tomita T, Iwatsubo T (2013) FTY720/fingolimod, a sphingosine analogue, reduces amyloid-β production in neurons. PLoS One 8:e64050

Xin C, Ren S, Pfeilschifter J, Huwiler A (2004) Heterologous desensitization of the sphingosine 1-phosphate receptors by purinoceptor activation in renal mesangial cells. Br J Pharmacol 143:581–589

Xin C, Ren S, Eberhardt W, Pfeilschifter J, Huwiler A (2006) The immunomodulators FTY720 and its phosphorylated derivative activate the Smad signaling cascade and upregulate connective tissue growth factor and collagen IV expression in renal mesangial cells. Br J Pharmacol 147:164–174

Yamamoto R, Okada Y, Hirose J, Koshika T, Kawato Y, Maeda M, Saito R, Hattori K, Harada H, Nagasaka Y, Morokata T (2014) ASP4058, a novel agonist for sphingosine 1-phosphate receptors 1 and 5, ameliorates rodent experimental autoimmune encephalomyelitis with a favorable safety profile. PLoS One 8:e110819

Zhang L, Wang HD, Ji XJ, Cong ZX, Zhu JH, Zhou Y (2013) FTY720 for cancer therapy (review). Oncol Rep 30:2571–2578

Zhou H, Murthy K (2004) Distinctive G protein-dependent signaling in smooth muscle by sphingosine 1-phosphate receptors $S1P_1$ and $S1P_2$. Am J Physiol 286: C1130–C1138

Zhou C, Ling MT, Lee TKW, Man K, Wang X, Wong YC (2006) FTY720, a fungus metabolite, inhibits invasion ability of androgen-independent prostate cancer cells through inactivation of RhoA-GTPase. Cancer Lett 223:36–47

Zhu J, Liu Y, Pi Y, Jia L, Wang L, Huang Y (2014) Systemic application of sphingosine 1-phosphate receptor 1 immunomodulator inhibits corneal allograft rejection in mice. Acta Opthalmol 92:e12–21

Sphingosine Kinase Activation Assay

Purpose and Rationale

Sphingosine 1-phosphate produced by two sphingosine kinase isoenzymes, denoted SphK1 and SphK2, is the ligand for a family of specific G-protein-coupled receptors that regulate cytoskeletal rearrangements and cell motility. Unlike the proliferative action of SphK1, the isoenzyme SphK2 has been shown to possess antiproliferative and proapoptotic action. Both kinases have been cloned and functionally characterized (Kohama et al. 1998; Liu et al. 2000, 2003; Nava et al. 2000; Olivera et al. 2000; Igarashi et al. 2003; Paugh et al. 2003; Sanchez et al. 2003; Billich et al. 2005; Döll et al. 2005; Hait et al. 2005; Kharel et al. 2005; Okada et al. 2005; De Palma et al. 2006; Zemann et al. 2006; Gao and Smith 2011; Neubauer and Pitson 2013; Tonellli et al. 2013; Zhang et al. 2013; Ceccom et al. 2014; Plano et al. 2014; Shen et al. 2014; Tamashiro et al. 2014; Tous et al. 2014). A recent summary of drugs in clinical trials targeting the sphingosine 1-phosphate pathway illustrates the potential roles of this axis in cancer and autoimmune inflammatory disease (Kunkel et al. 2013).

Sphingosine kinase activity assays were performed in a similar way by Paugh et al. (2003) and by Huwiler et al. (2006).

Procedure

Sphingosine Kinase Activity Assay

In vitro kinase reactions were performed according to Olivera et al. (2000). In brief, 30 µg of protein lysates was incubated with 50 µmol/l of sphingosine (dissolved as 1 mmol/l stock solution in 4 mg/ml of BSA in PBS) and 10 µCi (370 kBq) of [γ-^{32}P]ATP for 15 min at 37 °C. For SK-2 activity assay, the same buffer including 1 M KCl was used to inhibit SK-1 activity (Liu et al. 2000). Reactions were terminated by addition of 20 µl of 1 N HCl followed by 800 µl of chloroform/methanol/HCl (100:200:1, v/v), 240 µl of chloroform, and 240 µl of 2 mol/l KCl. After vigorous vortexing and phase separation, 50 µl of the lower organic phase was loaded onto

TLC plates and run in 1-butanol/ethanol/acetic acid/water (80:20:10:20, v/v).

Evaluation

Spots corresponding to S1P were analyzed and quantified using an imaging system (Fuji).

References and Further Reading

Billich A, Bornancin F, Mechtcheriakova D, Natt F, Huesken D, Baumruker T (2005) Basal and induced sphingosine kinase 1 activity in A549 carcinoma cells: function in cell survival and IL-1 β and TNF-α induced production of inflammatory mediators. Cell Signal 17:1203–1217

Ceccom J, Loukh N, Lauwers-Cances V, Touriol C, Nicaise Y, Gentil C, Uro-Coste E, Pitson S, Maurage CA, Duyckaerts C, Cuvillier O, Delisle M-B (2014) Reduced sphingosine kinase-1 and enhanced sphingosine 1-phosphate lyase expression demonstrate deregulated sphingosine 1-phosphate signaling in Alzheimer's disease. Acta Neuropathol Commun 2:1–10

De Palma C, Meacci E, Perrotta C, Bruni P, Clementi E (2006) Endothelial nitric oxide synthase activation by tumor necrosis factor a through neutral sphingomyelinase 2, sphingosine kinase 1, and sphingosine 1 receptors. Arterioscler Thromb Vasc Biol 26:99–105

Döll F, Pfeilschifter J, Huwiler A (2005) The epidermal growth factor stimulates sphingokinase-1 expression and activity in the human mammary carcinoma cell line MCF7. Biochim Biophys Acta 1738:72–81

Gao P, Smith CD (2011) Ablation of sphingosine kinase-2 inhibits tumor cell proliferation and migration. Mol Cancer Res 9:1509–1519

Hait NC, Sarkar S, Le Stunff H, Mikami A, Maceyka M, Milstien S, Spiegel S (2005) Role of sphingosine kinase 2 in cell migration toward epidermal growth factor. J Biol Chem 280:29462–29469

Huwiler A, Döll F, Ren S, Klawitter S, Greening A, Römer I, Bubnova S, Reinsberg L, Pfeilschifter J (2006) Histamine increases sphingosine kinase-1 expression and activity in the human endothelial cell line E.A. hy926 by a PCK-α-dependent mechanism. Biochim Biophys Acta 1761:367–376

Igarashi N, Okada T, Hayashi S, Fujita T, Jahangeer S, Nakamura SI (2003) Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis. J Biol Chem 278:46832–46839

Kharel Y, Lee S, Snyder AH, Sheasley-O'Neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KSK, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR (2005) Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. J Biol Chem 280:36856–36872

Kohama T, Olivera A, Edsall L, Nagiec MM, Dickson R, Spiegel S (1998) Molecular cloning and functional characterization of murine sphingosine kinase. J Biol Chem 273:23722–23728

Kunkel GT, Maceyka M, Milstien S, Spiegel S (2013) Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. Nat Rev Drug Discov 12:688–702

Liu H, Sugiura M, Nava VE, Edsall LC, Kono K, Poultoni S, Milstien S, Kohama T, Spiegel S (2000) Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform. J Biol Chem 275:19513–19520

Liu H, Toman RE, Goparaju SK, Maceyka M, Nava VE, Sankala H, Payne SG, Bektas M, Ishii I, Chun J, Milstien S, Spiegel S (2003) Sphingosine kinase 2 is a putative BH3-only protein that induces apoptosis. J Biol Chem 278:40330–40336

Nava VE, Lacana E, Poulton S, Liu H, Sugiura M, Kono K, Milstien S, Kohama T, Spiegel S (2000) Functional characterization of human sphingosine kinase-1. FEBS Lett 473:81–84

Neubauer HA, Pitson SM (2013) Roles, regulation and inhibitors of sphingosine kinase 2. FEBS J 280:5317–5336

Okada T, Ding G, Sonoda H, Kajimoto T, Haga Y, Khosrowbeygi A, Goa S, Miwa N, Jahangeer S, Nakamura SI (2005) Involvement of N-terminal-extended form of sphingosine kinase 2 in serum-dependent regulation of cell proliferation and apoptosis. J Biol Chem 280:36318–36325

Olivera A, Barlow KD, Spiegel S (2000) Assaying sphingosinekinase activity. Methods Enzymol 311:215–223

Paugh SW, Payne SG, Barbour SE, Milstien S, Spiegel S (2003) The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett 554:189–193

Plano D, Amin S, Sharma AK (2014) Importance of sphingosine kinase (SphK) as a target in developing cancer therapeutics and recent developments in the synthesis of novel SphK inhibitors. J Med Chem 57:5509–5524

Sanchez T, Estrada-Hernandez T, Paik JH, Wu MT, Venkataraman K, Brinkmann V, Claffey K, Hla T (2003) Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. J Biol Chem 278:27281–27290

Shen H, Giordano F, Wu Y, Chan J, Zhu C, Liosevic I, Wu X, Yao K, Chen B, Baumgart T, Sieburth D, de Camilli P (2014) Coupling between endocytosis and sphingosine kinase 1 recruitment. Nat Cell Biol 16:652–662

Tamashiro PM, Furuya H, Shimizu Y, Kawamori T (2014) Sphingosine kinase 1 mediates head and neck squamous cell carcinoma invasion through sphingosine 1-phosphate receptor 1. Cancer Cell Int 14:76

Tonellli F, Alossaimi M, Natarajan V, Gorshikova I, Berdyshev E, Bittman R, Watson DG, Pyne S, Pyne NJ (2013) The roles of sphingosine kinase 1 and 2 in regulating the metabolome and survival of prostate cancer cells. Biomolecules 3:316–333

Tous M, Ferrer-Lorente R, Badimon L (2014) Selective inhibition of sphingosine kinase-1 protects adipose tissue against LPS-induced inflammatory response in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 307:E437–446

Zemann B, Kinzel B, Müller M, Reuschel R, Mechtcheriakowa D, Urtz N, Bomancin F, Baumruker T, Billich A (2006) Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood 107:1454–1458

Zhang L, Urtz N, Gaertner F, Legate KR, Petzold T, Lorenz M, Mazharian A, Watson SP, Massberg S (2013) Sphingosine kinase 2 (Sphk2) regulates platelet biogenesis by providing intracellular sphingosine 1-phosphate (S1P). Blood 122:791–802

Lymphocyte Trafficking After Sphingosine 1-Phosphate Receptor Agonists

Purpose and Rationale

Adaptive immunity depends on T cell exit from the thymus and T and B cells traveling between secondary lymphoid organs to survey for antigen. After activation in lymphoid organs, T cells must again return to circulation to reach sites of infection. The immunomodulatory drug FTY720 induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing via the S1P receptor 1 (Chiba et al. 1998; Yanagawa et al. 1998a, b; Henning et al. 2001; Forrest et al. 2004; Matloubian et al. 2004; Hait et al. 2005; Kharel et al. 2005; Huwiler et al. 2006). Mandala et al. (2000) described alteration of lymphocyte trafficking by sphingosine 1-phosphate receptor agonists.

Procedure

Induction of Lymphopenia and Reduction of Thoracic Duct (TD) Lymphocytes by S1P and Analogues in Rats

Blood or thoracic duct lymph lymphocyte counts were determined by autoanalyzer (H2000, CARESIDE, Culver City, Calif., USA) and normalized to counts in vehicle controls after administration of FTY720 (2.5 mg/kg p.o.) or test compound. S1P was administered by continuous infusion beginning at 8 mg/kg/h for 20 min followed by 2 mg/kg/h for a further 220 min. The measured physiological S1P concentration in rat plasma by LC-MS was 0.5 μg/ml. This rose to a C_{max} of 2.5 μg/ml at 30 min and was maintained at 1.5 μg/ml for the remainder of the experiment. Studies on the effect on lymphocyte numbers in thoracic duct-cannulated rats were performed after the administration of FTY720 or test compound. Lymph flow remained constant

for the duration of the experiment, and numbers are shown as the average cell concentration maintained over the preceding 30 min.

FACS Measurement of Peripheral Blood Lymphocyte Depletion in Cannulated Rats

Percentage depletion by FTY720 compared to vehicle control was measured. Similar nadir lymphopenia was produced by FTY720 or non-metabolizable phosphonates. Peripheral blood samples were diluted 1:1 with phosphate-buffered saline (PBS), layered on the same volume of Lymphocyte Separation Medium (ICN Biomedicals, Aurora, Ohio, USA), and centrifuged at 400 g for 30 min. Peripheral blood mononuclear cells (PBMC) were resuspended in PBS and counted using a hemocytometer. PBMC were then stained with FITC-labeled anti-CD8, PE-labeled anti-CD45RA, and Cy-chrome-labeled anti-CD4 antibodies. Numbers of CD4-, CD8-, and CD45RA-positive cells were calculated by multiplying total PBMC count with the percentages of $CD4^+$, $CD8^+$, and $CD45RA^+$ generated from flow cytometry.

Quantitation of Lymph Node Cells

Single cell suspensions were prepared by passage of tissues through a 40-μm sieve. Peripheral blood lymphocytes were further isolated from spleens by ammonium chloride lysis of red blood cells. Cells were subsequently washed in UltraCULTURE medium (Biowhittaker, Walkersville, Md., USA), and all samples were adjusted to the same volume with PBS. An equal volume of 4 % paraformaldehyde was added while gently vortexing the samples. The total number of viable, unstained lymphocytes per sample was determined by flow cytometry (FACScan; Becton Dickinson) using CellQuest software (Becton Dickinson), based upon forward- and side-scatter characteristics. Beads (Sigma; P7458) were used as an internal standard.

Evaluation

Data were calculated as cell number per node by dividing the total number of lymphocytes quantitated by the number of nodes harvested per site (i.e., the number of Peyer's patches and mesenteric or peripheral lymph nodes collected).

Modifications of the Method

Kawa et al. (1997) reported inhibition of chemotactic motility and trans-endothelial migration of human neutrophils by sphingosine 1-phosphate.

Fueller et al. (2003) described activation of human monocytic cells by lysophosphatidic acid and sphingosine-1-phosphate.

Roviezzo et al. (2004) studied human eosinophil chemotaxis and selective in vivo recruitment by sphingosine 1-phosphate. Kunisawa et al. (2007) showed that sphingosine 1-phosphate may regulate peritoneal B cell trafficking and Thangada et al. (2010) using adoptive transfer experiments in wild-type mice, and mice mutated for the sphingosine 1-phosphate receptor showed that cell surface residency of the receptor determines the kinetics of lymphocyte egress. Yang et al. (2014) showed that fingolimod (FTY720) may prevent inflammation-sensitized hypoxic ischemia brain injury in newborn rats.

References and Further Reading

Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457

Chiba K, Yanagawa Y, Masubuchi Y, Karaoka H, Kawaguchi T, Ohtsuki M, Hoshino Y (1998) FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. J Immunol 160:5037–5044

Chiba K, Maeda Y, Seki N, Kataoka H, Sugahara K (2014) Role of sphingosine 1-phosphate (S1P) and effects of fingolimod, an S1P receptor 1 antagonist in lymphatic circulation and autoimmune disease. AIMS Mol Sci 1:162–182

Forrest M, Sun SY, Hajdu R, Bergstrom J, Card D, Doherty G, Hale J, Keohane C, Meyers C, Milligan J, Mills S, Nomura H, Rosen H, Rosenbach M, Shei GJ, Singer II, Tian M, West S, White V, Xie J, Proia RL, Mandala S (2004) Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate agonists in rodents are mediated via distinct receptor subtypes. J Pharmacol Exp Ther 309:758–768

Fueller M, Wang DA, Tigyi G, Siess W (2003) Activation of human monocytic cells by lysophosphatidic acid and sphingosine-1-phosphate. Cell Signal 15:367–375

Hait NC, Sarkar S, Le Stunff H, Mikami A, Maceyka M, Milstien S, Spiegel S (2005) Role of sphingosine kinase 2 in cell migration toward epidermal growth factor. J Biol Chem 280:29462–29469

Henning G, Ohl L, Junt T, Reiterer P, Brinkmann V, Nakano H, Hohenberger W, Lipp M, Förster R (2001) CC chemokine receptor 7-dependent and -independent pathways for lymphocyte homing: modulation by FTY720. J Exp Med 194:1875–1881

Huwiler A, Döll F, Ren S, Klawitter S, Greening A, Römer I, Bubnova S, Reinsberg L, Pfeilschifter J (2006) Histamine increases sphingosine kinase-1 expression and activity in the human endothelial cell line E.A. hy926 by a PCK-α-dependent mechanism. Biochim Biophys Acta 1761:367–376

Kawa S, Kimura S, Hakomori SI, Igarashi Y (1997) Inhibition of chemotactic motility and trans-endothelial migration of human neutrophils by sphingosine 1-phosphate. FEBS Lett 420:196–200

Kharel Y, Lee S, Snyder AH, Sheasley-O'Neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KSK, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR (2005) Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. J Biol Chem 280:36856–36872

Kimura T, Boehmler AM, Seitz G, Kuçi S, Wiesner T, Brinkmann V, Kanz L, Möhle R (2004) The sphingosine 1-phosphate receptor agonist FTY720 supports CXCR4-dependent migration and bone marrow homing of human $CD34^+$ progenitor cells. Blood 103:4478–4486

Kunisawa J, Kurashima Y, Gohda M, Higuchi M, Ishikawa I, Miura F, Ogahara I, Kiyono H (2007) Sphingosine 1-phosphate regulates peritoneal B-cell trafficking for subsequent intestinal IgA production. Blood 109:3749–3756

Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thronton R, Shei GJ, Card D, Keohane CA, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2000) Alteration of lymphocyte trafficking by sphingosine receptor agonists. Science 296:346–349

Matloubian M, Lo CG, Cinamom G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG (2004) Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 427:355–360

Roviezzo F, del Galdo F, Abbate G, Bucci M, D'Agostino B, Antunes E, de Dominicis G, Parente L, Rossi F, Cirino G, de Palma R (2004) Human eosinophil chemotaxis and selective in vivo recruitment by sphingosine 1-phosphate. Proc Natl Acad Sci U S A 101:11170–11175

Thangada S, Khanna KM, Blaho VA, Oo ML, Im D-S, Guo C, Lefrancois L, Hla T (2010) Cell-surface residence of sphingosine 1-phosphate receptor 1 on lymphocytes determines the lymphocyte egress kinetics. J Exp Med 207:1475–1483

Yanagawa Y, Sugahara K, Kataoka H, Kawaguchi T, Masubuchi Y, Chiba K (1998a) FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. II. FTY720 prolongs allograft survival by decreasing T cell infiltration into grafts but not cytokine production in vivo. J Immunol 160:5493–5499

Yanagawa Y, Masubuchi Y, Chiba K (1998b) FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. Immunology 95:591–594

Yang D, Sun Y-Y, Bhaumik SK, Li Y, Baumann JM, Lin X, Zhang Y, Lin S-H, Dunn RS, Liu C-Y, Shie F-S, Lee Y-H, Wills-Karp M, Chougnet CA, Kallapur SG, Lewkowich AP, Lindquist DM, Murali-Krishna K, Kuan C-Y (2014) Blocking lymphocyte trafficking with FTY720 prevents inflammation-sensitized hypoxic-ischemic brain injury in newborns. J Neurosci 34:16467–15481

Zemann B, Kinzel B, Müller M, Reuschel R, Mechtcheriakowa D, Urtz N, Bomancin F, Baumruker T, Billich A (2006) Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood 107:1454–1458

In Vivo Methods for Testing Immunological Factors

Spontaneous Autoimmune Diseases in Animals

Several spontaneous autoimmune diseases have been reported in several inbred animal strains:

New Zealand black mouse (NZB mouse) (Bielschowski et al. 1959; Howie and Helyer 1968; Barthold et al. 1974; Blanchard and Bach 1980). The NZB mouse develops a spontaneous autoimmune disease with autoimmune hemolytic anemia, splenomegaly, glomerulonephritis, lymphoproliferative disorders, and peptic ulcerations.

New Zealand black/white F1 (B/W) mouse (Helyer and Howie 1963; Kessler 1968). These animals develop nephritis similar to that in human systemic lupus erythematosus and show mononuclear cell infiltration in salivary and lachrymal glands such as in human Sjögren's syndrome.

A substrain of the autoimmune-prone mouse, NZB/kl, was found to show spontaneous elevation of the auditory brainstem response threshold with age (Sone et al. 1995).

Immunodeficient alymphoplasia mice were recommended as a spontaneous model for Sjögren's syndrome (Tsubata et al. 1996). Mice homozygous for an autosomal-recessive mutation aly (alymphoplasia) lack both lymph nodes and Peyer's patches and show defects in both humoral and cellular immunity. Histopathological analyses revealed chronic inflammatory changes in exocrine organs such as the salivary gland, the lacrimal gland, and the pancreas.

The **Palmerston North autoimmune mouse strain** which exhibits both spontaneous systemic autoimmune disease and otic capsule bone formation has been proposed as a model for otic capsule osteogenesis and otosclerosis (Hertler and Trune 1990; Traynor et al. 1992).

In aging **BDF1 mice**, Hayashi et al. (1988) described spontaneous development of autoimmune sialadenitis.

Robison et al. (1994) examined the relationship between orchitis and aspermatogenesis in various strains of H_2 congenic mice and defined a genetic predisposition to spontaneous aspermatogenesis.

Motheaten mice. Mice homozygous for the autosomal-recessive motheaten (me) or the allelic viable motheaten (me^v) mutations develop severe and early-age onset of systemic autoimmune and inflammatory disease (Green and Shultz 1975; Shultz et al. 1984; Shultz 1988; Su et al. 1998).

The genetic, hormonal, and behavioral influence on spontaneously developing arthritis in normal mice has been reviewed by Holmdahl et al. (1992).

Nonobese diabetic mouse (NOD mouse) (Makino et al. 1980; Miyazaki et al. 1985; Leiter et al. 1987). The inbred NOD mouse is considered a good model for type I diabetes mellitus. Mononuclear cells infiltrate the pancreatic islets of Langerhans from 6 to 8 weeks of age, followed by a progressive and selective destruction of insulin-producing β-cells and the onset of IDDM from the 12th week of age onwards.

Itoh et al. (1997) studied the requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice.

Quartey-Papafio et al. (1995) showed that aspartate at position 57 of nonobese diabetic I-A (g7) β-chain diminishes the spontaneous incidence of insulin-dependent diabetes mellitus in the NOD mouse.

The NOD mouse was also recommended to study the pathogenesis of autoimmune thyroiditis (Many et al. 1996; Giarratana et al. 2007).

Inherited inflamed joints. Adipue et al. (2011) established a new spontaneous murine model of inflammatory arthritis of inherited inflamed joints (IIJ) established from AR mice that appeared in a 5B6 transgenic mouse-breeding colony.

Qi et al. (2013) developed a murine model of spontaneous liver disease resembling autoimmune hepatitis, and Yang et al. (2014) developed a murine model of spontaneous peripheral polyneuropathy.

Bio-breeding rat (BB rat) (Like et al. 1982; Field 1983; Yale and Marliss 1984). On the basis of clinical and histopathological parameters, the BB rat is considered a useful model for human IDDM. The disease in the BB rat is characterized by infiltration of lymphocytes and macrophages into the islets of Langerhans.

Allen and Thupari (1995) described spontaneous autoimmune lymphocytic thyroiditis in *BB/Wor rats.*

Obese strain chicken (OS chicken) (van Tienhoven and Cole 1962; Cole 1966; Cole et al. 1968, 1970; Wick et al. 1974). The OS chicken is perhaps the best studied model for an organ-specific, spontaneously occurring autoimmune disease, viz., spontaneous autoimmune thyroiditis, which closely resembles human Hashimoto thyroiditis. The spontaneous autoimmune thyroiditis in obese chicken was further studied by Neu et al. (1986), Kroemer et al. (1989), Cihak et al. (1995), Hala et al. (1996), and Dietrich et al. (1997).

Chickens of the University of California line 200 **(UCD-200 chickens)** develop an inherited inflammatory fibrotic disease that closely resembles human progressive systemic sclerosis (scleroderma) (Gershwin et al. 1981; Van de Water et al. 1984; Brezinscheck et al. 1993).

Schumm-Draeger and Fortmeyer (1996) described **autoimmune thyroiditis in the cat** as a spontaneous disease model.

Spontaneous autoimmune thyroiditis was found in **Mastomys** (*Praeomys coucha*) by Solleveld et al. (1985) and recommended as an animal model of human disease.

References and Further Reading

Adipue IA, Wilcox JT, King C, Rice CAY, Shaum KM, Suard CM, ten Brink E, Miller SD, McMahon EJ (2011) Characterisation of a novel and spontaneous mouse model of inflammatory arthritis. Arthritis Res Ther 13:R1114

Allen EM, Thupari JN (1995) Thyroglobulin-reactive T lymphocytes in thyroiditis-prone BB/Wor rats. J Endocrinol Invest 18:45–49

Barthold DR, Kysela S, Steinberg AD (1974) Decline in suppressor T cell function with age in female NZB mice. J Immunol 112:9

Bielschowski M, Helyer BJ, Howie JB (1959) Spontaneous anemia in mice of the NZB/BL strain. Proc Univ Otago Med Sch 37:9–11

Blanchard D, Bach MA (1980) Thymic function in NZB mice. Clin Exp Immunol 42:1–9

Brezinscheck HP, Gruschwitz M, Sgone R, Moormann S, Herold M, Gershwin ME, Wick G (1993) Effects of cytokine application on glucocorticoid secretion in an animal model for systemic scleroderma. J Autoimmun 6:719–733

Cihak J, Hoffmann-Fezer G, Koller A, Kaspers B, Merkle H, Hala K, Wick G, Losch U (1995) Preferential TCR Vβ1 gene usage by autoreactive T cells in spontaneous autoimmune thyroiditis of the obese strain of chickens. J Autoimmun 8:507–520

Cole RK (1966) Hereditary hypothyroidism in domestic fowl. Genetics 13:1021–1033

Cole RK, Kite JH, Witebsky E (1968) Hereditary autoimmune thyroiditis in the fowl. Science 160:1357–1358

Cole RK, Kite JH, Wick G, Witebsky E (1970) Inherited autoimmune thyroiditis in the fowl. Poult Sci 49:480–488

Del Prete GF, Tiri A, Parronchi P, Pinchera A, Romagnani S, Ricci M, Mariotti S (1989) Thyroiditis as a model of organ specific autoimmune disease. Clin Exp Rheumatol 7(Suppl 3): S41–S46

Dietrich HM, Oliveira Dos Santos AJ, Wick G (1997) Development of spontaneous autoimmune thyroiditis in Obese strain (OS) chickens. Vet Immunol Immunopathol 57:141–146

Field JB (ed) (1983) The juvenile diabetes foundation workshop on the spontaneously diabetic BB rat: its potential for insight into human juvenile diabetes. Metabolism 32(Suppl 1): 1–166

Gershwin ME, Abplanalp JJ, Castles RM, Ikeda J, van de Water J, Eklund J, Haynes D (1981) Characterization of a spontaneous disease of white leghorn chickens resembling progressive systemic sclerosis (scleroderma). J Exp Med 153:1640–1659

Giarratana N, Penna G, Adorini L (2007) Animal models of spontaneous autoimmune disease: type 1 diabetes in the non-obese diabetic mouse. Methods Mol Biol 380:285–311

Green MC, Shultz LD (1975) Motheaten, an immunodeficient mutant of the mouse. I. Genetics and pathology. J Hered 66:250–258

Hala K, Malin G, Dietrich H, Loesch U, Boeck G, Wolf H, Kaspers B, Geryk J, Falk M, Boyd RL (1996) Analysis of the initiation period of spontaneous autoimmune thyroiditis (SAT) in the obese strain (OS) of chickens. J Autoimmun 9:129–138

Hayashi Y, Kurashima C, Utsuyama M, Hirokawa K (1988) Spontaneous development of autoimmune sialadenitis in aging BDF1 mice. Am J Pathol 132:173–179

Helyer BW, Howie JB (1963) Renal disease associated with positive lupus erythematosus test in a cross-bred strain of mice. Nature 197:197

Hertler CK, Trune DR (1990) Otic capsule bony lesions in the Palmerston North autoimmune mouse. Otolaryngol Head Neck Surg 103:713–718

Holmdahl R, Jansson L, Andersson M, Jonsson R (1992) Genetic, hormonal and behavioural influence on spontaneously developing arthritis in normal mice. Clin Exp Immunol 88:467–472

Howie JB, Helyer BJ (1968) The immunology and pathology of NZB mice. Adv Immunol 9:215–266

Itoh N, Imagawa M, Hanafusa T, Waguri M, Yamamoto K, Iwahshi A, Morikawi M, Nakajima H, Miyagawa J, Namba M, Makino S, Nagata S, Kono N, Matsuzawa Y (1997) Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. J Exp Med 186:613–618

Kessler HS (1968) A laboratory model for Sjögren's syndrome. Am J Pathol 52:671–685

Kroemer G, Neu N, Kuehr T, Dietrich F, Fassler R, Hala K, Wick G (1989) Immunogenetic analysis of spontaneous autoimmune thyroiditis of obese strain of chickens. Clin Immunol Immunopathol 52:202–213

Leiter EH, Prochazka M, Coleman DL (1987) Animal model of human disease. The non-obese diabetic (NOD) mouse. Am J Pathol 128:380–383

Like AA, Butler L, Williams RM, Appel MC, Weringer EJ, Rossini AA (1982) Spontaneous autoimmune diabetes mellitus in the BB rat. Diabetes 31(Suppl):7–13

Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y (1980) Breeding of a non-obese, diabetic strain of mice. Exp Anim 29:1–13

Many MC, Maniratunga S, Denef JF (1996) The non-obese diabetic (NOD) mouse: an animal model for autoimmune thyroiditis. Exp Clin Endocrinol Diabetes 104(Suppl 3):17–20

Miyazaki A, Hanafusa T, Yamada K, Miyagawa J, Fujino-Kurihara H, Nagajima H, Nonaka K, Tarui S (1985) Predominance of T lymphocytes in pancreatic islets and spleen of pre-diabetic non-obese diabetic (NOD) mice: a longitudinal study. Clin Exp Immunol 60:622–630

Neu N, Hala K, Dietrich H, Wick G (1986) Genetic background of spontaneous autoimmune thyroiditis in the obese strain of chickens studied in hybrids with an inbred line. Int Arch Allergy Appl Immunol 80:168–173

Qi N, Liu P, Zhang Y, Wu H, Chen Y, Han D (2013) Development of a spontaneous liver disease resembling autoimmune hepatitis in mice lacking tyro3, axl and mer receptor tyrosine kinases. PLoS One 8:e66604

Quartey-Papafio R, Lund T, Chandler P, Picard J, Ozegbe P, Hutchings PR, O'Reilly L, Kioussis D, Simpson E, Cooke A (1995) Aspartate at position 57 of nonobese diabetic

I-A (g7) β-chain diminishes the spontaneous incidence of insulin-dependent diabetes mellitus. J Immunol 154:5567–5575

Robison R, Tung KSK, Meeker ND, Monson FG, Teuscher C (1994) A murine model of spontaneous aspermatogenesis: linkage to H_2. J Reprod Immunol 26:251–260

Schumm-Draeger PM, Fortmeyer HP (1996) Autoimmune thyroiditis – spontaneous disease models – cat. Exp Clin Endocrinol Diabetes 104(Suppl 3):12–13

Schuurs AHWM, Verheul HAM, Wick G (1989) Spontaneous autoimmune models. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 449–485

Shultz LD (1988) Pleiotropic effects of deleterious alleles in the "motheaten" locus. Curr Top Microbiol Immunol 137:216–222

Shultz LD, Coman DR, Bailey CL, Beamer WG, Sidman CL (1984) "Viable motheaten," a new allele in the motheaten locus. Am J Pathol 116:179–192

Solleveld HA, Coolen J, Haajiman JJ (1985) Animal model of human disease: autoimmune thyroiditis. Spontaneous autoimmune thyroiditis in praomys (mastomys) coucha. Am J Pathol 119:345–349

Sone M, Nariuchi H, Saito K, Yanagita M (1995) A substrain of NZB mouse as an animal model of autoimmune inner ear disease. Hear Res 83:26–26

Su X, Zhou T, Yang P, Edwards CK III, Mountz JD (1998) Reduction of arthritis and pneumonitis in motheaten mice by soluble tumor necrosis factor receptor. Arthritis Rheum 41:139–149

Traynor SJ, Cohen JI, Morton JI, Trune DR (1992) Immunohistochemical analysis of otic capsule osteogenesis in the Palmerston North autoimmune mouse. Otolaryngol Head Neck Surg 106:196–201

Tsubata R, Tsubata T, Hiai H, Shinkura R, Matsumura R, Sumida T, Miyawaki S, Ishida H, Kumagai S, Nakao I, Honjo T (1996) Autoimmune disease of exocrine organs in immunodeficient alymphoplasia mice: a spontaneous model for Sjøgren's syndrome. Eur J Immunol 26:2742–2748

Van de Water J, Gershwin ME, Aplanalp H, Wick G, van der Mark K (1984) Serial observations and definition of mononuclear cell infiltrates in avian scleroderma, an inherited fibrotic disease of chickens. Arthritis Rheum 27:807–815

van Tienhoven A, Cole RK (1962) Endocrine disturbance in obese chickens. Anat Rev 142:111–122

Wick G, Sundick RS, Albini B (1974) The obese strain (OS) of chickens: an animal model with spontaneous autoimmune thyroiditis. Clin Immunol Immunopathol 3:272–300

Yale JF, Marliss EB (1984) Altered immunity and diabetes in the BB rat. Clin Exp Immunol 57:1–11

Yang M, Rainone A, Shi XQ, Fournier S, Zhang J (2014) A new model of spontaneous autoimmune peripheral polyneuropathy: implications for Guillain-Barre syndrome. Acta Neuropath Commun 2:5. doi: 10.1186/2051-5960-2-5

Acute Systemic Anaphylaxis in Rats

Purpose and Rationale

Rats are immunized with ovalbumin and *Bordetella pertussis* suspension as adjuvant. After 11 days, the animals are challenged by intravenous injection of ovalbumin. The shock symptoms can by inhibited by corticosteroids and intravenous disodium cromoglycate.

Procedure

Female Sprague–Dawley rats weighing 120 g are immunized by i.m. injection of 10 mg/kg highly purified ovalbumin. Simultaneously 1 ml of *Bordetella pertussis* suspension (2×10^{10} organisms) is injected intraperitoneally. IgE antibodies are induced and attached to the surface of mast cells and basophilic granulocytes. Eleven days later, the animals are challenged by intravenous injection of 25 mg/kg highly purified ovalbumin. This results in the formation of antigen–antibody complexes on the surface of mast cells and basophilic granulocytes in blood and in all organs with immediate release of various mediators of anaphylaxis, such as histamine, serotonin, SRS-A,

and prostaglandins; in shock symptoms; and 80 % lethality. Corticosteroids, e.g., dexamethasone 1–10 mg/kg s.c., are given 18 h prior to challenge or 30 mg/kg disodium cromoglycate i.v. before injection of ovalbumin. Ten to 20 animals are used for each group.

Evaluation

The shock symptoms are scored and mortality counted. Results after treatment are compared with untreated controls. Pretreatment with corticosteroids or disodium cromoglycate can inhibit death and ameliorate shock symptoms. Statistical calculation is performed using the χ^2-test.

Modifications of the Method

Desensitization by repeated "microshocks" of constant strength in guinea pigs has been reported by Herxheimer (1952).

Acute systemic anaphylaxis experiments have also been performed in guinea pigs and in mice. In guinea pigs, anaphylactic bronchospasm can be measured with the Konzett and Rössler method (Davies and Evans 1973).

Moreover, anaphylactic bronchospasm can be measured in isolated guinea pig lungs according to the method of Bhattacharya and Delaunois (1955).

Anaphylaxis can be measured in the chopped guinea pig lung by assay of the supernatant in the isolated guinea pig ileum in the presence of 2×10^{-7} M atropine (Austen and Brocklehurst 1961).

Ufkes and Ottenhof (1984) sensitized Brown Norway rats with a suspension of trinitrophenyl-haptenized ovalbumin together with $AlPO_4$ as adjuvant. Bronchial and cardiovascular functions were studied after treatment with antiallergic agents and antigen challenge.

Elwood et al. (1992) studied the effect of dexamethasone and cyclosporine A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown Norway rats.

References and Further Reading

Austen KF, Brocklehurst WE (1961) Anaphylaxis in chopped guinea pig lung. J Exp Med 113:521–537

Bhattacharya BK, Delaunois AL (1955) An improved method for the perfusion of isolated lung of guinea pig. Arch Int Pharmacodyn Ther 101:495–510

Davies GE, Evans DP (1973) Studies with two new phosphodiesterase inhibitors (ICI 58,301 and ICI 63,197) on anaphylaxis in guinea pigs, mice and rats. Int Arch Allergy 45:467–478

Elwood W, Lötvall JO, Barnes PJ, Chung KF (1992) Effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown-Norway rats. Am Rev Respir Dis 145:1289–1294

Herxheimer H (1952) Repeatable 'microshocks' of constant strength in guinea pig anaphylaxis. J Physiol 117:251–255

Omote M, Sakai K, Mizusawa H (1994) Acute effects of deflazacort and its metabolite 21-desacetyl-deflazacort on allergic reactions. Arzneim Forsch/Drug Res 44:149–153

Ufkes JGR, Ottenhof M (1984) Characterization of various anti-allergic agents using a new method for inducing systemic anaphylaxis in the rat. J Pharmacol Methods 11:219–226

Anti-anaphylactic Activity (Schultz–Dale Reaction)

Purpose and Rationale

Guinea pigs are sensitized against egg albumin. Challenge after 3 weeks causes in isolated organs' release of mediators, e.g., histamine, which induce contraction in isolated ileum.

Procedure

Guinea pigs of either sex weighing 300–350 g are sensitized with alum-precipitated egg albumin. Alum egg albumin is prepared by dissolving egg albumin (1 mg/ml) in 6 % aluminum hydroxide gel, suspended in saline. The mixture is stirred and kept at room temperature. Each animal receives at the same time injections of 0.125 ml of this mixture in each foot pad and 0.5 ml subcutaneously. After 4 weeks, the animals are killed and the ileum is dissected out. Cleaned pieces, about 2–3 cm long, are mounted in an organ bath containing Tyrode solution at 37 °C. The strips are allowed

to equilibrate for 15 min. The contractility of the ileum strips is tested by adding 10^{-4} g/ml $BaCl_2$ solution. To one organ bath the standard (2×10^{-6} g/ml final concentration of tribenoside = 1-*O*-ethyl-3,5,6-tri-*O*-benzyl-D-glucofuranoside = Glyvenol CIBA) and to other vials the test compounds (final concentration up to 10^{-5} g/ml) are added. One organ bath serves as control. After 3 min, ovalbumin in a final concentration of 2×10^{-6} g/ml is added. The contractions are recorded with strain gauges by a polygraph.

Evaluation

The results are expressed as presence or absence of blocking activity (percentage inhibition). If anti-anaphylactic activity is observed, ED_{50} values using different doses are calculated.

Critical Assessment of the Method

Positive results can also be achieved with spasmolytics, local anesthetics, antihistaminics, and sympathicomimetics.

Modifications of the Method

The method has been modified by testing histamine release in the lung after challenging with egg albumin. Either lung strips from sensitized guinea pigs are suspended in an organ bath and their contractions are measured after addition of egg albumin or the entire lung tissue is dissected out and washed free from blood by perfusing with warm oxygenated Tyrode solution via the pulmonary artery. The lung tissue is chopped and washed with Tyrode solution in order to remove the remaining blood. The chopped lung tissue is divided into 24 samples, each of approximately 100 mg wet weight. These are incubated at 37 °C in Tyrode solution for 15 min with continuous agitation by rocking, after which 1 mg/ml of egg albumin is added to the reaction mixture. After shaking for 10 min at 37 °C, the supernatant is collected and assayed for histamine with guinea pig ileum. Atropine sulfate 2 mg/ml is added in Tyrode solution. The residual histamine is obtained by boiling the tissue in 5 ml Tyrode solution for 10 min. The tubes are then placed on ice for 1 h to allow complete diffusion. Released histamine is expressed as a percentage of total histamine content.

Koppel et al. (1981) developed a method to induce contraction of immunologically sensitized mouse trachea by antigen (Schultz–Dale reaction).

The trachea of sensitized guinea pigs was used by Omote et al. (1994). Choi et al. (2008) measured the effects of dehydroepiandrosterone on the Schultz–Dale reaction and the Th2 immune response in sensitized BALB/c mice. Guhathakurta et al. (2013) determined the effects of UNIM-352 and Naik et al. (2013) the effects of extract of Zizyphus jujuba fruits, both natural products, in a rodent model of systemic anaphylaxis.

References and Further Reading

Anderson P, Brattsand R (1982) Protective effects of the glucocorticoid, budesonide, on lung anaphylaxis in actively sensitized guinea pigs: inhibition of the IgE – but not of the IgG – mediated anaphylaxis. Br J Pharmacol 76:139–147

Austen KF, Brocklehurst WE (1961) Anaphylaxis in chopped guinea pig lung. I. Effect of peptidase substrates and inhibitors. J Exp Med 113:521–537

Choi IS, Cui Y, Koh YA, Cho YB, Won YH (2008) Effects of dehydroepiandrosterone on the Schultz-Dale reaction and the Th2 immune response in sensitized BALB/c mice. Korean J Asthma Allergy Clin Immunol 28:121–127

Dale HH (1913) The anaphylactic reaction of plain muscle in the guinea-pig. J Pharmacol Exp Ther 4:167–223

Guhathakurta S, Gulati K, Rai N, Banerji BD, Jamil SS, Ray A (2013) An experimental study to evaluate the anti-inflammatory and immunomodulatory effects of UNIM-352, a polyherbal preparation for bronchial asthma. Med Plant Res 3:3–12

Koppel GA, Haisch KD, Spaethe SM, Schmidtke JR, Fleisch JH (1981) Schultz–Dale reaction in mouse trachea. J Pharmacol Methods 6:39–43

Laekeman GM, Herman AG, van Nueten JM (1977) Influence of different drugs on the slow response of the intestine during the

Schultz–Dale reaction. Arch Int Pharmacodyn Ther 230:335
Naik SR, Bhagatb S, Shaha PD, Tarea AA, Ingawalea D, Wadekara RR (2013) Evaluation of anti-allergic and anti-anaphylactic activity of ethanolic extract of *Zizyphus jujuba* fruits in rodent. Rev Bras Farm 23:811–818
Omote M, Sakai K, Mizusawa H (1994) Acute effects of deflazacort and its metabolite 21-desacetyl-deflazacort on allergic reactions. Arzneim Forsch/Drug Res 44:149–153
Schultz WH (1910) Physiological studies in anaphylaxis. 1. The reaction of smooth muscle of the guinea-pig sensitized with horse serum. J Pharmacol Exp Ther 1:549–567

Passive Cutaneous Anaphylaxis

Purpose and Rationale

Passive cutaneous anaphylaxis is an immune reaction of the immediate type. By passive immunization of rats in the skin with rat anti-ovalbumin serum and a challenge 2 days later with ovalbumin at the same skin area, antigen–antibody complexes are formed in the mast cells inducing release of mediators. This results in vasodilatation, increase in permeability of the vessel walls, and leakage of plasma. To make the allergic reaction visible, Evan's blue dye is administered along with the antigen. Evan's blue dye is attached to the albumin fraction of plasma, producing a blue spot. This blue spot indicates that an anaphylactic reaction has taken place in the skin.

Procedure

For preparation of antiserum, male rats weighing 200–250 g are adrenalectomized and are allowed to recover for 3 days. Thereafter, animals are sensitized with egg albumin (1 mg/animal) using aluminum hydroxide gel (200 mg) as adjuvant. Alum egg albumin is prepared by dissolving 1 mg/ml of egg albumin in 20 % aluminum hydroxide gel, suspended in saline. Each animal simultaneously receives 0.125 ml of the above solution in each foot pad and 0.5 ml subcutaneously. After 8 days, the animals are bled and antiserum is collected.

For the test, the antiserum is diluted in such a manner as to give a wheal of 15–20 mm diameter in a preliminary titration. Aliquots of 100 µl of appropriate dilution of antiserum are injected intradermally into the shaved dorsal skin of normal male rats weighing about 100 g. After 24 h of latent period, each animal is challenged with the intravenous administration of 0.1 ml of 2.5 % Evans blue dye containing 25 mg/ml of egg albumin. In the case of intravenous administration, the test compound is administered simultaneously with the antigen and the dye. In case of oral testing, the compound is given orally 1 h prior to challenge. The animals are sacrificed 30 min after the challenge. The amount of Evans blue dye leaked at the site of passive cutaneous anaphylactic reaction is extracted and determined colorimetrically at 620 µm wavelength.

Evaluation

The amount of Evans blue extracted from passive cutaneous anaphylactic reaction is taken as 100 %. Percent inhibition of passive cutaneous anaphylactic reaction in the rats treated with the test compound is calculated. The standard disodium cromoglycate at a dose of 3 mg/kg i.v. or 30 mg/kg orally results in 80–100 % inhibition. Using different doses, ED_{50} values can be calculated.

Modifications of the Method

Goose and Blair (1969) used *Bordetella pertussis* and extracts of the worm *Nippostrongylus brasiliensis* as antigens in passive cutaneous anaphylaxis experiments in the rat.

Patterson et al. (1971) tested passive cutaneous reactivity to antihuman IgE in rhesus monkeys.

Without immunization, plasma extravasation after bradykinin injection can be tested in anesthetized Sprague–Dawley rats (Lembeck et al. 1991). Evans blue dye is injected to stain plasma proteins. After injection of bradykinin antagonists followed by bradykinin injection, the rats are perfused with physiological saline. The trachea, the urinary bladder, and the duodenum are resected, weighed, and incubated for 48 h in formamide at 50 °C (Saria et al. 1983). The

amount of Evans blue extracted is measured photometrically at 620 nm.

Vascular reactions to histamine, histamine liberator, and leukotaxine in the skin of guinea pigs using pontamine sky blue 6× as indicator were studied by Miles and Miles (1952). Babakin et al. (2008) investigated the effects of fullerene-60 in both systemic and both rat and murine passive cutaneous models of anaphylaxis, and Zhu et al. (2009) showed that the proteinase-activated receptor 2 is involved in passive cutaneous murine model of anaphylaxis and that it can be inhibited by tacrolimus.

Hitomi et al. (2010) discovered that mice deficient in the immunoglobulin-like receptor Allergin-1 developed enhanced passive systemic and cutaneous anaphylaxis, and Han et al. (2013) showed that the phytoalexin resveratrol inhibited both IgE-mediated basophilic mast cell degranulation and passive cutaneous anaphylaxis in a murine model.

References and Further Reading

Babakin AA, Andrievsky G, DuBuske LM (2008) Inhibition of systemic and passive cutaneous anaphylaxis by water-soluble Fullerene 60. J Allergy Clin Immunol 123:S118

Goose J, Blair AMJN (1969) Passive cutaneous anaphylaxis in the rat, induced with two homologous reagin-like antibodies and its specific inhibition with disodium cromoglycate. Immunology 16:749–760

Griesbacher T, Lembeck F (1987) Actions of bradykinin antagonists on bradykinin-induced plasma extravasation, venoconstriction, prostaglandin E_2 release, nociceptor stimulation and contraction of the iris sphincter muscle of the rabbit. Br J Pharmacol 92:333–340

Han S-Y, Bae J-Y, Park S-H, Kim Y-H, Park JHY, Kang Y-H (2013) Resveratrol inhibits IgE-mediated basophilic mast cell degranulation and passive cutaneous anaphylaxis in mice. J Nutr. doi: 10.3945/jn.112.173302

Hitomi K, Tahara-Hanaoka S, Someya S, Fujiki A, Tada H, Sugiyama T, Shibayama S, Shibuya K, Shibuya A (2010) An immunoglobulin-like receptor, allergin-1, inhibits immunoglobulin E-mediated immediate hypersensitivity reactions. Nat Immunol 11:601–607

Katayama S, Shionoya H, Ohtake S (1975) A new simple method for extraction of extravasated dye in the skin. Jpn J Pharmacol Suppl 25:103P

Lembeck F, Griesbacher T, Eckhardt M, Henke S, Breipohl G, Knolle J (1991) New, long-acting, potent bradykinin antagonists. Br J Pharmacol 102:297–304

Miles AA, Miles EM (1952) Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea pigs. J Physiol 118:228–257

Patterson R, Talbot CH, Brandfonbrener M (1971) The use of IgE mediated responses as a pharmacologic test system. The effect of disodium cromoglycate in respiratory and cutaneous reactions and in the electrocardiograms of rhesus monkeys. Int Arch Allergy Immunol 41:592–603

Saria A, Lundberg JM, Skofitsch G, Lembeck F (1983) Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, histamine and by antigen challenge. Naunyn Schmiedeberg's Arch Pharmacol 324:212–218

Watanabe N, Ovary Z (1977) Antigen and antibody detection by in vivo methods: a reevaluation of passive cutaneous anaphylactic reactions. J Immunol Methods 14:381–390

Zhu Y, Peng C, Xu JG, Liu YX, Zhu QG, Liu JY, Li FQ, Wu JH, Hu JH (2009) Participation of proteinase-activated receptor-2 in passive cutaneous anaphylaxis-induced scratching behaviour and the inhibitory effect of tacrolimus. Biol Pharm Bull 32:1173–1176

Arthus-Type Immediate Hypersensitivity

Purpose and Rationale

The immune complex-induced Arthus reaction comprises inflammatory factors that have been implicated in the acute responses in joints of rheumatic patients. Complement and polymorphonuclear neutrophils are activated via precipitating antigen–antibody complexes leading to an inflammatory focus characterized by edema,

hemorrhage, and vasculitis. Arthus reaction of the immediate type becomes maximal 2–8 h after the challenge.

Procedure

Ovalbumin Suspension

1,700 mg ovalbumin is suspended in 100 ml paraffin oil. 4.38 ml pertussis vaccine is suspended in 70 ml 0.9 % NaCl solution. Both suspensions are mixed to form an emulsion.

Wistar or Sprague–Dawley rats of either sex weighing 220–280 g can be used. Seven days prior to the start of the experiment, rats are sensitized by i.m. administration of 0.5 ml of the ovalbumin suspension. They are housed in groups of eight with standard food and water ad libitum.

Twenty-four hours and 1 h prior to induction of the Arthus reaction, test compounds are administered to groups of eight animals. The rats are challenged by injection of 0.1 ml of 0.04 % solution of highly purified ovalbumin in the left hind paw. Swelling of the paw occurs which reaches a maximum after a few hours. The footpad thickness can be measured by calipers. One group of sensitized animals treated with solvent alone serves as positive control; one group of non-sensitized animals treated with solvent alone serves as negative control. Standard doses are 30 mg/kg cortisone or 10 mg/kg prednisolone p.o.

Evaluation

The change in footpad thickness is expressed as the percent change from the vehicle control group. Comparison of experimental group to positive control is evaluated statistically using Student's *t*-test.

Modifications of the Method

Instead of ovalbumin, sheep red blood cell suspensions can be used for immunization and for challenge in mice (Omote et al. 1994).

Nagakawa et al. (1990) sensitized mice by s.c. injection of bovine serum albumin in complete Freund's adjuvant and boosted on day 21 by an intradermal injection of BSA. On day 28, the Arthus reaction was elicited by intradermal injection of BSA. Four hours later, an erythematous skin reaction over an area of more than 8 mm^2 was regarded as positive.

Kamei et al. (1991) immunized guinea pigs by injection of a mixture of egg albumin and Freund's complete adjuvant subcutaneously into the food pad or i.m. into the hind leg. The injection was repeated four times at 7-day interval. Ten days after the last immunization, 0.2 ml of 2.5 % egg albumin was injected sc. into the dorsal skin of the animals. The intensity of the Arthus reaction was evaluated by measuring the inflamed area according to scores.

References and Further Reading

Bartlett RR, Gebert U, Kerékjártó B, Schleyerbach R, Thorwart W, Weitmann KU (1989) Substituted 3-phenyl-7H-thiazolo (3,2-b)(1,2,4) triazin-7-ones as antiinflammatory agents with immunomodulating properties. Drugs Exp Clin Res 15:521–526

Horvat J, Vidic B, Kosec D, Stojic Z, Jankovic BD (1990) Suppression of Arthus and delayed hypersensitivity reactions to bovine serum albumin by dopaminergic antagonists. Period Biol 92:81–82

Kamei C, Izushi K, Adachi Y, Shimazawa M, Tasaka K (1991) Inhibitory effect of epinastine on the type II–IV allergic reactions in mice, rats and guinea pigs. Arzneim Forsch/Drug Res 41:1150–1153

Nagakawa Y, Ogawa T, Kobayashi M, Wagatsuma K, Munakata H, Umezu K, Sato S, Shibata Y, Inoue K, Ishida N (1990) Immunopharmacological studies of 4-acetylaminophenyl-acetic acid. (MS-932). Int J Immunother 6:131–140

Omote M, Sakai K, Mizusawa H (1994) Acute effects of deflazacort and its metabolite 21-desacetyl-deflazacort on allergic reactions. Arzneim Forsch/Drug Res 44:149–153

Delayed-Type Hypersensitivity (DTH)

Purpose and Rationale

Delayed-type hypersensitivity is a reaction of cell-mediated immunity and becomes visible only

after 16–24 h. The same methods as for testing immediate-type hypersensitivity can be used.

Procedure

Rats are sensitized in the same way by i.m. administration of 0.5 ml ovalbumin suspension 7 days prior to the start of the experiment as described for testing immediate-type hypersensitivity. They are challenged by injection of 0.1 ml of 0.04 % solution of highly purified ovalbumin in the left hind paw. Footpad thickness is measured immediately and 24 h after ovalbumin administration.

Modifications of the Method

Mizukoshi et al. (1994) injected female CDF1 mice intradermally with a suspension of 2×10^8 sheep red blood cells/50 μl into the left foot pad. A second booster of the same dose was given to the right foot pad on day 4. The thickness of the foot pads was measured on the following day, and the difference in the thickness between the right and the left food pads was taken as the degree of swelling.

Kamei et al. (1991) immunized mice by applying 0.15 ml of 7 % picryl chloride/ethanol solution to the skin of the shaved abdomen. The second immunization was performed 6 days later. One week after the second immunization, 1 drop of 1 % picryl chloride olive oil solution was applied to the ear, and the thickness of the ear was measured by a thickness gauge 24 h later.

Heriazon et al. (2009) investigated the induction of DTH and interferon gamma to Candida albicans and anti-hen egg white lysozyme antibody as phenotypic markers of enhance bovine immune response, and their studies suggest that this combination of test antigens could be used as phenotypic markers of immune responsiveness in cattle. Escandell et al. (2010) investigated the inhibition of DTH by the plant product cucurbitacin R which was shown to reduce human T lymphocyte proliferation.

Yang et al. (2011) used the DTH model to a three-protein cocktail with that of a purified protein derivative, and Atkinson et al. (2012) extended the model to study the similarities with collagen-induced arthritis and human rheumatoid arthritis.

References and Further Reading

Atkinson SM, Usher PA, Kvist PH, Markholst H, Haase C, Nansen A (2012) Establishment and characterization of a sustained delayed-type hypersensitivity model with arthritic manifestations in C57/BL6J mice. Arthritis Res Ther 14:R134

Borel JF (1989) Pharmacology of cyclosporine (Sandimmune). IV. Pharmacological properties in vivo. Pharmacol Rev 41:259–371

Borel JF, Feurer C, Magnée C, Stähelin H (1977) Effects of the new anti-lymphocytic peptide cyclosporin A in animals. Immunology 32:1017–1025

Escandell JM, Recio M-C, Giner RM, Manez S, Cerda-Nicolas M, Merfort I, Rios J-L (2010) Inhibition of delayed-type sensitivity by cucurbitacin R through the curbing of lymphocyte proliferation and cytokine expression by means of nuclear factor AT translocation to the nucleus. J Pharmacol Exp Ther 232:352–363

Heriazon A, Yager JA, Sears W (2009) Mallard BA (2009) Induction of delayed-type hypersensitivity and interferon-gamma to Candida albicans and anti-hen-egg white lysozyme antibody as phenotypic markers of enhance bovine immune response. Vet Immunol Immunopathol 129:93–100

Herrmann P, Schreier MH, Borel JF, Feurer C (1988) Mast cell degranulation as a major event in the effector phase of delayed-type hypersensitivity induced by cloned helper cells. Int Arch Allergy Appl Immunol 86:102–105

Kamei C, Izushi K, Adachi Y, Shimazawa M, Tasaka K (1991) Inhibitory effect of epinastine on the type II–IV allergic reactions in mice, rats and guinea pigs. Arzneim Forsch/Drug Res 41:1150–1153

Li F, Song D, Lu Y, Zhu H, Chen Z, He X (2013) Delayed-type hypersensitivity (DTH) immune response related with EBV-DNA in nasopharyngeal carcinoma treated with autologous dendritic cell vaccination after radiotherapy. J Immunother 36:208–214

Malajian D, Belsito DV (2013) Cutaneous delayed-type hypersensitivity in patients with

atopic dermatitis. J Am Acad Dermatol 69:232–237
Mizukoshi S, Tsukamoto M, Tanaka H, Nakamura K, Kato F (1994) Antiinflammatory and immunosuppressive effects of 1,6-anhydro-3,4-dideoxy-2-furfuryl-*β*-D-*threo*-3-enopyranose (MT 2221), a novel anhydro-enopyranose derivative, on experimental animal models. Biol Pharm Bull 17:1070–1074
Nagakawa Y, Ogawa T, Kobayashi M, Wagatsuma K, Munakata H, Umezu K, Sato S, Shibata Y, Inoue K, Ishida N (1990) Immunopharmacological studies of 4-acetylaminophenyl-acetic acid. (MS-932). Int J Immunother 6:131–140
Pence BD, Lowder TW, Keylock KT, Potter VJV, Cook MD, McAuley E, Woods JA (2012) Relationship between systemic inflammation and delayed-type hypersensitivity response to Candida antigen in older adults. PLoS One 7: e36403
Schindewolf M, Gobst C, Kroll H, Recke A, Louwen F, Wolter M, Kaufmann R, Boehncke W-H, Lindhoff-Last E, Ludwig RJ (2013) High incidence of heparin-induced allergic delayed-type hypersensitivity reactions in pregnancy. J Allergy Clin Immunol 132:131–139
Titus RG, Chiller JM (1981) A simple and effective method to assess murine delayed type hypersensitivity to proteins. J Immunol Methods 45:65–78
Yang H, Troudt J, Grover A, Arnett K, Lucas M, Cho YS, Bielefeldt-Ohmann H, Talor J, Izzo A, Dobos KM (2011) Three protein cocktails mediate delayed-type hypersensitivity responses indistinguishable from that elicited by purified protein derivative in the guinea pig model of *Mycobacterium tuberculosis* infection. Infect Immun 79:716–723

Reversed Passive Arthus Reaction

Purpose and Rationale

In the reversed passive Arthus reaction, the antigen is injected intravenously followed by a local injection – either intradermally or into the pleural space – of the respective antibody. Generation of an immune-mediated reverse passive Arthus reaction in the rat pleural cavity results in a classic acute inflammatory response. The methods are used to evaluate new anti-inflammatory agents.

Procedure

Male Lewis rats weighing 200–250 g are fasted overnight prior to use with free access to water. The animals receive 5 mg bovine serum albumin in 0.2 ml sterile saline intravenously, followed 30 min later by injection of 1 mg rabbit anti-BSA in 0.2 ml sterile saline into the right pleural cavity under light halothane anesthesia. Drugs or vehicle controls are administered by gastric gavage in 1 ml/100 g body weight at different times prior to the anti-BSA. The animals are sacrificed at various intervals after anti-BSA injections by CO_2 inhalation (after 5 min for thromboxane B_2 determination, after 10 min for leukotriene B_4 determination, and after 4 h at the peak time of neutrophil infiltration). The fluid exudate is removed from the pleural cavity by gentle vacuum aspiration and the volume is recorded. Eicosanoids in the pleural exudate are quantitated by commercial RIA kits.

Evaluation

The values after treatment with various doses of test compounds are compared with those of vehicle controls.

Modifications of the Method

The antibody can be injected intradermally into the shaved skin of rats after intravenous injection of the antigen (e. g., human albumin) together with Evans blue dye solution. Extravasated dye is determined in skin punches (Camussi et al. 1990; Burch et al. 1992; Okamoto et al. 1992).

Bailey and Sturm (1983) induced the reverse passive Arthus reaction in rats using bovine serum albumin as antigen into the tail vein and rabbit anti-bovine serum albumin into the skin site. One hour after oral dosing with vehicle or drug, animals were lightly anesthetized and their hair was shaved from the middorsal region with electric clippers. Each animal was injected intradermally with 40 µl on the left side of the middorsal line and

with 40 µl of rabbit anti-bovine serum albumin (5.0 mg/ml antibody protein), diluted 1:4 with phosphate-buffered saline on the right side of the dorsal midline. Immediately following the intradermal challenge, each rat received 0.5 ml phosphate-buffered saline containing 1.0 mg bovine serum albumin injected in the tail vein. Four hours after intradermal challenge, the animals were sacrificed. The full-thickness skin was removed from the back, and disks 8 mm in diameter were punched out with a metal punch. Wet weight of the samples from the phosphate-buffered saline- and antibody-injected site was determined, and the edema induced by the reverse passive Arthus reaction calculated as the difference between both weights.

References and Further Reading

Bailey PJ, Sturm A (1983) Immune complexes and inflammation. A study of the activity of anti-inflammatory drugs in the reverse passive Arthus reaction in the rat. Biochem Pharmacol 32:475–481

Berkenkopf JW, Weichman BM (1991) Comparison of several new 5-lipoxygenase inhibitors in a rat Arthus pleurisy model. Eur J Pharmacol 193:29–34

Berkenkopf JW, Marinari LR, Weichman BM (1991) Phospholipase A_2 acyl-hydrolytic activity in rat RPAR-induced pleurisy. Agents Actions 34:93–96

Burch RM, Connor JR, Bator JM, Weitzberg M, Laemont K, Noronha-Blob L, Sullivan JP, Steranka LR (1992) NPC 15669 inhibits the reverse passive Arthus reaction in rats by blocking neutrophil recruitment. J Pharmacol Exp Ther 263:933–937

Camussi G, Tetta C, Bussolino F, Baglioni C (1990) Antiinflammatory peptides (antiflammins) inhibit synthesis of platelet-activating factor, neutrophil aggregation and chemotaxis, and intradermal inflammatory reactions. J Exp Med 171:913–927

Carter GW, Young PR, Albert DH, Bouska J, Dyer R, Bell RL, Summers JB, Brooks DW (1991) 5-Lipoxygenase inhibitory activity of Zileuton. J Pharmacol Exp Ther 256:929–937

Chang YH, Otterness IG (1981) Effects of pharmacologic agents on the reversed passive Arthus reaction in the rat. Eur J Pharmacol 69:155–164

Humphrey JH (1955a) The mechanism of Arthus reactions. I. The role of polymorphonuclear leukocytes and other factors in reversed passive Arthus reactions in rabbits. Br J Exp Pathol 36:268–282

Humphrey JH (1955b) The mechanism of Arthus reactions. II. The role of polymorphonuclear leukocytes and platelets in reversed passive Arthus reactions in the guinea-pig. Br J Exp Pathol 36:283–289

Kim KH, Martin IC, Young PR, Carter GW, Haviv F (1990) Inhibitors of immune complex-induced inflammation: 5-substituted 3-[1-(2-benzoxazolyl) hydrazino]propanenitrile derivatives. J Pharm Sci 79:682–684

Okamoto H, Iwahisa Y, Terawasa M (1992) Suppression of the Arthus reaction by Y-24180, a potent and specific antagonist of platelet-activating factor. Agents Actions 35:149–158

Ting PC, Kaminski JJ, Sherlok MH, Tom WC, Lee JF, Bryant RW, Watnick AD, McPhail AT (1990) Substituted 1,3-dihydro-2*H*-pyrrolo[2,3-*b*] pyridin-2-ones as potential antiinflammatory agents. J Med Chem 33:2697–2706

Yamamoto S, Dunn CD, Deporter DA, Capasso F, Willoughby DA, Huskisson EC (1975) A model for the quantitative study of Arthus (immunologic) hypersensitivity in rats. Agents Actions 5:374–377

Adjuvant Arthritis in Rats

Purpose and Rationale

Adjuvant arthritis in rats has been described by Pearson and Wood (1959) exhibiting many similarities to human rheumatoid arthritis. Injections of complete Freund's adjuvant into the rat paw induce inflammation as primary lesion with a maximum after 3–5 days. Secondary lesions occur after a delay of approximately 11–12 days which are characterized by inflammation of non-injected sites (hindleg, forepaws, ears, nose, and tail) and a decrease of weight and immune responses. The procedure has been modified by

several authors in order to differentiate between anti-inflammatory and immunosuppressive activity (e.g., Perper et al. 1971). Anti-inflammatory compounds do not inhibit secondary lesions, which are prevented or diminished by immunosuppressive agents. Two protocols, termed "preventative" (or "prophylactic") and "therapeutic" (or "established") adjuvant arthritis, have gained wide usage for assessing a drug's potential anti-arthritic activity (Schorlemmer et al. 1999).

Procedure

The choice of the animal strain has been found to be very important for the performance of this test. Wistar–Lewis rats have been proven to be very suitable in contrast to other substrains. Male rats with an initial body weight of 130–200 g are used. On day 1, they are injected into the suplantar region of the left hind paw with 0.1 ml of complete Freund's adjuvant. This consists of 6 mg mycobacterium butyricum (Difco) being suspended in heavy paraffin oil (Merck) by thoroughly grinding with mortar and pestle to give a concentration of 6 mg/ml. Dosing with the test compounds or the standard is started on the same day and continued for 12 days. Paw volumes of both sides and body weight are recorded on the day of injection, whereby paw volume is measured plethysmographically with equipment as described in the paw edema tests. On day 5, the volume of the injected paw is measured again, indicating the primary lesion and the influence of therapeutic agents on this phase. The severity of the induced adjuvant disease is followed by measurement of the non-injected paw (secondary lesions) with a plethysmometer. Purposely, from day 13–21, the animals are not dosed with the test compound or the standard. On day 21, the body weight is determined again, and the severity of the secondary lesions is evaluated visually and graded according the following scheme:

		Score
Ears	Absence of nodules and redness	0
	Presence of nodules and redness	1
Nose	No swelling of connective tissue	0
	Intensive swelling of connective tissue	1
Tail	Absence of nodules	0
	Presence of nodules	1
Forepaws	Absence of inflammation	0
	Inflammation of at least one joint	1
Hind paws	Absence of inflammation	0
	Slight inflammation	1
	Moderate inflammation	2
	Marked inflammation	3

(*continued*)

Evaluation

(a) For primary lesions: The percent inhibition of paw volume of the injected left paw over vehicle control is measured at day 5.
(b) For secondary lesions: The percentage inhibition of paw volume of the non-injected right paw over controls is measured at day 21.
(c) An arthritic index is calculated as the sum of the scores as indicated above for each animal. The average of the treated animals is compared with the control group.
(d) The total percentage change is calculated as follows by addition of:

Percent inhibition of the injected paw on day 5 + percent inhibition of the non-injected paw on day 21 + percent change of the arthritic index.

Doses of 0.3 mg/kg indomethacin p.o. and 20–50 mg/kg phenylbutazone p.o. are effective on the primary lesions when dosage is started at the day of injection of the irritant. They are not effective on the secondary lesions.

In contrast, immunosuppressants like cyclophosphamide at a dose of 7 mg/kg inhibited the secondary lesions even when started at day 9 or later.

Critical Assessment of the Method

Evidence was given that adjuvant arthritis in the rat is associated with chronic pain (Colpaert 1987). The measure of pain in this model still presents some technical problems since the evaluation is based on the somewhat biased observation of the behavioral responses.

Modifications of the Method

A review was given by Gardner (1960) on the experimental production of arthritis.

Moran et al. (1999) compared adjuvant arthritis and selected animal models of arthritis to rheumatoid arthritis with special emphasis on the mechanism of joint destruction.

Kazuna and Kawai (1975) and Rooks et al. (1982) used rats with established lesions to test analgesics in the arthritic flexion pain test. The method is claimed to be specific by detecting only central analgesics and nonsteroidal anti-inflammatory drugs but not other classes such as CNS-depressant or antihistaminic drugs.

Brackertz et al. (1977) established antigen-induced arthritis in the mouse by immunization with methylated bovine serum albumin in complete Freund's adjuvant with B pertussis vaccine.

A streptococcal cell wall-induced arthritis in rats has been described by Wilder et al. (1982, 1987) and Yocum et al. (1986).

Lewis et al. (1997) studied degradation of articular cartilage in a rat monoarthritis model induced by an intra-articular injection of *Propionibacterium acnes*.

Crossley et al. (1989) reported on a monoarticular antigen-induced arthritis in rabbits and mice.

α-2-Glycoprotein levels have been recommended as parameter for severity and inhibition of experimental immunoarthritis in the rat by Sandow et al. (1971).

Pircio et al. (1975) recommended a method for the evaluation of analgesic activity using adjuvant-induced arthritis in rats. The degree of vocalization was recorded from five rats placed together in a counting chamber.

Cruwys et al. (1994) sensitized rats on day 0 and 7 with multiple intradermal injections of methylated bovine serum albumin emulsified in Freund's complete adjuvant. On day 21, the animals were challenged by the intra-articular injection of 100 µl 0.5 % solution of methylated bovine serum albumin into the right knee. The progress of the monoarticular arthritis was monitored by daily measurement of joint diameter.

Butler et al. (1991) described a limited arthritic pain model for chronic pain and inflammation studies using injections of 0.05 ml of complete Freund adjuvant into the left tibiotarsal joint of Sprague–Dawley rats.

Issekutz et al. (1994) studied the role of tumor necrosis factor-alpha and IL-1 in polymorphonuclear leukocyte and T lymphocyte recruitment to joint inflammation in adjuvant arthritis.

Esser et al. (1995) measured radiographic changes in adjuvant-induced arthritis in rats by quantitative image analysis. Digitized radiographs of the calcaneus were examined for changes in the mean and in the distribution of gray values. Periostal new bone formation was measured as an increase in image area of the calcaneus.

Mercuric chloride ($HgCl_2$) induces a syndrome of autoimmunity in Brown Norway rats characterized by a variety of IgG antibodies; very high concentrations of serum IgE, proteinuria, leukocytoclastic vasculitis which predominantly affects the cecum; and an inflammatory polyarthropathy (Kiely et al. 1995, 1996).

Kawahito et al. (2000) reported that 15-deoxy-$\Delta^{12,14}$-PGJ_2 which activates PPAR-α induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. Cuzzocrea et al. (2002) found that prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 attenuates the development of acute and chronic inflammation.

Bolon et al. (2004) described a method for rapid quantification of intralesional osteoclasts in the hind paws of Lewis rats with adjuvant-induced arthritis. A 4-µm-thick section of the decalcified hind paw was stained to demonstrate osteoclasts using an indirect immunoperoxidase method and a rabbit antihuman monoclonal antibody directed against the osteoclast marker cathepsin K, which is an osteoclast protease primarily responsible for the resorption of bone. The sections were evaluated using tiered, semiquantitative criteria to grade bone erosions and intralesional osteoclasts.

Kong et al. (1999), Campagnuolo et al. (2002), and Bolon et al. (2002a, b) used Lewis rats with adjuvant arthritis to describe the effects of osteoprotegerin, an endogenous antiosteoclast factor for protecting bone in rheumatoid arthritis.

Francischi et al. (2000) described anti-inflammatory and analgesic effects of the phosphodiesterase 4 inhibitor rolipram in the rat model of adjuvant-induced arthritis.

Boyle et al. (2001) reported anti-inflammatory effects of a non-nucleoside adenosine kinase inhibitor in rat adjuvant arthritis.

Fujisawa et al. (2002) demonstrated the effects of highly water-soluble matrix metalloproteinase inhibitors in a rat adjuvant-induced arthritis model.

Wei et al. (2004) described the effects and mechanisms of a dual inhibitor of interleukin-1 and tumor necrosis factor on adjuvant arthritis in rats.

Boe et al. (1999) reported that interleukin 6 knockout **mice** are resistant to antigen-induced experimental arthritis.

Gauldie et al. (2004) described a robust model of adjuvant-induced chronic unilateral arthritis in two mouse strains. DBA/1 and C57BL/6 male mice were injected intra-articularly into a stifle joint with FCA (5 μg in 5 μl) once per week for 4 weeks. Measurements of joint diameter and joint histopathology were used to monitor the course of arthritis. Inflammatory hyperalgesia was assessed as the pressure causing a limb withdrawal. Standard drugs, such as indomethacin or prednisolone, caused a decrease in joint inflammation and associated hyperalgesia.

Kim and Moudgil (2009) reviewed the genetic and other determinants of both susceptibility and resistance to adjuvant-induced arthritis in the rat, and Snekhalatha et al. (2013) conduced a detailed characterization of adjuvant-induced arthritis in the rat model comparing thermography, radiological imaging, and histopathology, a work extended by Vollmer et al. (2014) who used near-infrared fluorescence imaging to monitor the progress of experimental-induced arthritis in several rat models.

The adjuvant-induced arthritis model has been used to profile the activity of a number of candidate drugs which include DHOH, p38 and JAK inhibitors (Balague et al. 2012), bee venom (Darwish et al. 2013), peptides from heat shock protein 65 (Shi et al. 2014), and the saponin astragaloside IV (Wang 2014).

Consden et al. (1971), Cooke and Jasin (1972), Cooke et al. (1972), and Jasin and Cooke (1977) produced a chronic experimental monoarthritis by intra-articular injection of antigens into previously immunized **rabbits**.

Henderson et al. (1990) induced monoarticular arthritis in ovalbumin-sensitized rabbits by intra-articular injection of ovalbumin (antigen-induced arthritis) or in naive rabbits by injecting hyaluronic acid mixed with the polycation poly-D-lysine (polycation-induced arthritis).

Arner et al. (1995) compared the alterations in proteoglycan metabolism in antigen-induced arthritis and polycation-induced arthritis in rabbits and determined the involvement of interleukin-1 in the cartilage degradation that occurs in these models of rheumatoid arthritis.

Lewthwaite et al. (1995) studied the antifibrotic action of interleukin-1 receptor antagonist in antigen-induced monoarticular arthritis in New Zealand white rabbits.

Arthritis occurs in **pigs** due to infection with *Erysipelothrix rhusiopathiae* (Ajmal 1969). Experimental erysipelothrix infection in pigs can be used as a model for rheumatism research (Schulz et al. 1975a, b, 1977). Infections are established by oral or parenteral administration of standardized serotype B erysipelas strains.

Erysipelothrix arthritis could also be produced in rats and **rabbits** (White et al. 1975; Glynn 1977).

Arthritis due to infection with *Mycoplasma synoviae* occurs naturally among domestic poultry (Olson et al. 1954, 1964). Arthritis in **chickens** after mycoplasma infection has been used as experimental model (Kerr and Olson 1970; Cullen 1977).

Experimental models of arthritis due to streptococcal infections have been proposed for various species: **mice** (Cayeux et al. 1966; Hook et al. 1960; Ohanian et al. 1969), **rats** (Jasmin 1967; Koga et al. 1973), **rabbits** (Cecil et al. 1939; Cook and Fincham 1966; Ginsburg et al. 1968, 1977; Norlin 1960; Shimizu et al. 1958; Stein et al. 1973), and **pigs** (Roberts et al. 1968, 1969).

Avridine-Induced Arthritis

The injection of avridine [*N*,*N*-dioctadecyl-*N*′, *N*′-bis (2-hydroxyethyl) propanediamine/CP-20961], emulsified in Freund's adjuvant, at the base of the tail is arthritogenic in susceptible rat strains (Meacock et al. 1994; Brun et al. 1995;

Vingsbo et al. 1995; Lorentzen and Klareskog 1997; Joe and Wilder 1999; Van Bilsen et al. 2004).

References and Further Reading

Ajmal M (1969) Erysipelothrix rhusiopathiae and spontaneous arthritis in pigs. Res Vet Sci 10:579

Arner EC, Harris RR, DiMeo TM, Collins RC, Galbraith W (1995) Interleukin-1 receptor antagonist inhibits proteoglycan breakdown in antigen induced but not in polycation induced arthritis in the rabbit. J Rheumatol 22:1338–1346

Balague C, Pont M, Prats N, Godessart N (2012) Profiling of dihydroorotate dehydrogenase, p38 and JAK inhibitors in the rat adjuvant-induced arthritis model. Br J Pharmacol 166:1320–1332

Bartlett RR, Schleyerbach R (1985) Immunopharmacological profile of a novel isoxazol derivative, HWA 486, with potential antirheumatic activity. I. Disease modifying action on adjuvant arthritis of the rat. Int J Immunopharmacol 7:7–18

Beck FWJ, Whitehouse MW, Pearson CM (1974) Drug sensitivity of rat adjuvant arthritis, induced with 'adjuvants' containing no mineral oil components. Proc Soc Exp Biol Med 146:665–669

Boe A, Baiocchi M, Carbonatto M, Papoian R, Serlupi-Crescenzi O (1999) Interleukin 6 knock-out mice are resistant to antigen-induced experimental arthritis. Cytokine 11:1057–1064

Bolon B, Shalhoub V, Kostenuik PJ, Campagnuolo G, Morony S, Boyle WJ, Zack D, Feige U (2002a) Osteoprotegerin, an endogenous antiosteoclast factor for protecting bone in rheumatoid arthritis. Arthritis Rheum 46:3121–3135

Bolon B, Campagnuolo G, Feige U (2002b) Duration of bone protection by a single osteoprotegerin injection in rats with adjuvant-induced arthritis. Cell Mol Life Sci 59:1569–1576

Bolon B, Morony S, Cheng Y, Hu YL, Feige U (2004) Osteoclast numbers in Lewis rats with adjuvant-induced arthritis: identification of preferred sites and parameters for rapid quantitative analysis. Vet Pathol 41:30–36

Boyle DL, Kowaluk EA, Jarvis MF, Lee CH, Bhagwat SS, Williams W, Firestein GS (2001) Anti-inflammatory effects of ABT-702, a novel non-nucleoside adenosine kinase inhibitor, in rat adjuvant arthritis. J Pharmacol Exp Ther 296:495–500

Brackertz D, Mitchell GF, MacKay IR (1977) Antigen-induced arthritis in mice. Arthritis Rheum 20:841–850

Brun JG, Haland G, Haga HJ, Fagerhol MK, Jonsson R (1995) Effect of calprotectin in avridine-induced arthritis. APMIS 103:233–240

Butler SH, Godefroy F, Besson JM, Weil-Fugazza J (1991) Increase in "pain sensitivity" induced by exercise applied during the onset of arthritis in a model of monoarthritis in the rat. Int J Tissue React 13:299–304

Campagnuolo G, Bolon B, Feige U (2002) Kinetics of bone protection by recombinant osteoprotegerin therapy in Lewis rats with adjuvant arthritis. Arthritis Rheum 46:1926–1936

Cayeux P, Panijel J, Cluzan R, Levillain R (1966) Streptococcal arthritis and cardiomyopathy experimentally induced in white mice. Nature 212:688–691

Cecil RL, Angevine DM, Rothbard S (1939) Experimental arthritis in rabbits produced by streptococci and other organisms. Am J Med Sci 198:463–475

Colpaert FC (1987) Evidence that adjuvant arthritis in the rat is associated with chronic pain. Pain 28:201–222

Connolly KM, Stecher VJ, Danis E, Pruden DJ, LaBrie T (1988) Alteration of interleukin-1 production and the acute phase response following medication of adjuvant arthritic rats with cyclosporin-A or methotrexate. Int J Immunopharmacol 10:717–728

Consden R, Doble A, Glynn LE, Nind AP (1971) Production of a chronic arthritis with albumin. Its retention in rabbit knee joints. Ann Rheum Dis 30:307–315

Cook J, Fincham WJ (1966) Arthritis produced by intra-articular injection of streptolysin S in rabbits. J Pathol Bacteriol 99:283–297

Cooke TD, Jasin HE (1972) The pathogenesis of chronic inflammation in experimental antigen-induced arthritis. I. The role of antigen on the local immune response. Arthritis Rheum 15:327–337

Cooke TD, Hurd ER, Ziff M, Jasin HE (1972) The pathogenesis of chronic inflammation in experimental antigen-induced arthritis. II. Preferential localization of antigen-antibody complexes to collagenous tissues. J Exp Med 135:323–338

Crossley MJ, Holland T, Spowage M, Hunneyball IM (1989) Monarticular antigen-induced arthritis in rabbits and mice. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 415–439

Cruwys SC, Garrett NE, Perkins MN, Blake DR, Kidd BL (1994) The role of bradykinin B_1 receptors in the maintenance of intra-articular plasma extravasation in chronic antigen-induced arthritis. Br J Pharmacol 113:940–944

Cullen GA (1977) Mycoplasma infection and arthritis in chickens. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 240–255

Cuzzocrea S, Wayman NS, Mazzon E, Dugo L, di Paola R, Serraino I, Britti D, Chatterjee PK, Caputi AP, Thiemermann C (2002) The cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 attenuates the development of acute and chronic inflammation. Mol Pharmacol 61:997–1007

Darwish SF, El-Bakly WM, Arafa HM, El-Demerdash E (2013) Targeting TNF-a and NF-kB activation by bee venom: role in suppressing adjuvant induced arthritis and methotrexate hepatotoxicity in rat. PLoS One 8:e79284

del Pozo E, Graeber M, Payne T (1990) Regression of bone and cartilage loss in adjuvant arthritic rats after treatment with cyclosporin A. Arthritis Rheum 33:247–252

Esser RE, Hildebrand AR, Angelo RA, Watts AM, Murphey MD, Baugh LE (1995) Measurement of radiographic changes in adjuvant-induced arthritis in rats by quantitative image analysis. Arthritis Rheum 38:129–138

Francischi JN, Yokoro CM, Poole S, Tafuri WL, Cunha FQ, Teixeira MM (2000) Anti-inflammatory and analgesic effects of the phosphodiesterase 4 inhibitor rolipram in a rat model of arthritis. Eur J Pharmacol 399:243–249

Fujisawa T, Igeta K, Odake S, Morita Y, Yasuda J, Morikawa T (2002) Highly-water soluble matrix metalloproteineases inhibitors and their effects in a rat adjuvant-induced arthritis model. Bioorg Med Chem 10:2569–2581

Gardner DL (1960) The experimental production of arthritis. A review. Ann Rheum Dis 19:297–317

Gauldie SD, McQueen DS, Clarke CJ, Chessell IP (2004) A robust model of adjuvant-induced chronic unilateral arthritis in two mouse strains. J Neurosci Methods 139:281–291

Ginsburg I, Silberstein Z, Spira G, Bentwich Z, Boss JH (1968) Experimental arthritis in rabbits induced by group A streptococcal products. Experientia (Basel) 24:256–257

Ginsburg I, Zor U, Floman Y (1977) Experimental models of streptococcal arthritis: pathogenic role of streptococcal products and prostaglandins and their modification by anti-inflammatory agents. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 256–299

Glynn LE (1977) Erysipelothrix arthritis in rabbits. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 238–239

Henderson B, Pettipher ER, Murphy G (1990) Metalloproteinases and cartilage proteoglycan depletion in chronic arthritis. Comparison of antigen-induced and polycation-induced arthritis. Arthritis Rheum 33:241–246

Hook EW, Wagner RR, Lancefield RC (1960) An epizootic in Swiss mice caused by a group A streptococcus, newly designed type 50. Am J Hyg 72:11–119

Issekutz AC, Meager A, Otterness I, Issekutz TB (1994) The role of tumor necrosis factor-alpha and IL-1 in polymorphonuclear leukocyte and T lymphocyte recruitment to joint inflammation in adjuvant arthritis. Clin Exp Immunol 97:26–32

Jasin HE, Cooke TD (1977) Persistence of antigen in experimental allergic monoarthritis. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 28–32

Jasmin G (1967) Experimental arthritis in rats. A comprehensive review with specific reference to mycoplasma. In: Rohstein J (ed) Rheumatology, vol 1. Karger, Basel, pp 107–131

Joe B, Wilder RL (1999) Animal models of rheumatoid arthritis. Mol Med Today 5:367–369

Kawahito Y, Kondo M, Tsubouchi Y, Hashiramoto A, Bishop-Bailey D, Inoue KI, Kohno M, Yamada R, Hla T, Sano H (2000) 15-deoxy-$\Delta^{12,14}$-PGJ_2 induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. J Clin Invest 106:189–197

Kazuna S, Kawai K (1975) Evaluation of analgesic agents in rats with adjuvant arthritis. Chem Pharm Bull (Tokyo) 23:1184–1191

Kerr KM, Olson NO (1970) Pathology of chickens inoculated experimentally or contact-infected with *Mycoplasma synoviae*. Avian Dis 14:291–320

Kiely PDW, Thiru S, Oliveira DGB (1995) Inflammatory polyarthritis by mercuric chloride in the Brown Norway rat. Lab Invest 73:284–293

Kiely PDW, O'Brien D, Oliveira DGB (1996) Anti-CD8 treatment reduces the severity of inflammatory arthritis, but not vasculitis, in mercuric chloride-induced autoimmunity. Clin Exp Immunol 106:280–285

Kim EY, Moudgil KD (2009) The determinants of susceptibility/resistance to adjuvant arthritis in rats. Arthritis Res Ther 11:239–248

Koga T, Pearson CM, Narita T, Kotani S (1973) Polyarthritis induced in the rat by cell walls from several bacteria and two Streptomyces species. Proc Soc Exp Biol N Y 143:824–827

Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li JI, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM (1999) Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402:304–309

Leisten JC, Gaarde WA, Scholz W (1990) Interleukin-6 serum levels correlate with footpad swelling in adjuvant-induced arthritic Lewis rats treated with cyclosporin A or indomethacin. Clin Immunol Immunopathol 56:108–115

Lewis EJ, Bishop J, Bottomley KM, Bradshaw D, Brewster M, Broadhurst MJ, Brown PA, Budd JM, Elliott L, Greenham AK, Johnson WH, Nixon JS, Rose F, Sutton B, Wilson K (1997) Ro 32–3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. Br J Pharmacol 121:540–546

Lewthwaite J, Blake S, Thompson RC, Hardingham TE, Henderson B (1995) Antifibrotic action of interleukin-1 receptor antagonist in lapine monoarticular arthritis. Ann Rheum Dis 54:591–596

Lorentzen JC, Klareskog L (1997) Comparative susceptibility of DA, LEW, and LEW.1AV1 rats to arthritis induced by different arthritogens: mineral oil, mycobacteria, muramyl dipeptide avridine and rat collagen II. Transplant Proc 29:1692–1693

Meacock SC, Brandon DR, Billigham ME (1994) Arthritis in Lewis rats induced by the non-immunogenic adjuvant CP20961: an immunohistochemical analysis of the developing disease. Ann Rheum Dis 53:653–658

Mohr W, Wild A (1976) Adjuvant arthritis. Arzneim Forsch/Drug Res 26:1860–1866

Moran EL, Bogoch ER (1999) Animal models of rheumatoid arthritis. In: An YH, Friedman RJ (eds) Animal models in orthopaedic research. CRC Press LLC, Boca Raton, pp 369–390

Norlin G (1960) Experimental rheumatoid arthritis in rabbits. Acta Rheumatol Scand 6:309–319

Ohanian SH, Schwab JH, Cromartie WJ (1969) Relation to rheumatic-like lesions of the mouse to localization of group A streptococci cell walls. J Exp Med 129:37–49

Olson NO, Bletner JK, Shelton DC, Munro DA, Anderson GC (1954) Enlarged joint condition in poultry caused by an infectious agent. Poultry Sci 33:1075–1080

Olson NO, Kerr KM, Cambell A (1964) Control of infectious synovitis. The antigen study of three strains. Avian Dis 8:209–215

Pearson CM (1956) Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc Soc Exp Biol Med 91:95–101

Pearson CM (1963) Experimental joint disease. Observations on adjuvant-induced arthritis. J Chronic Dis 16:863–874

Pearson CM, Wood FD (1959) Studies on polyarthritis and other lesions induced in rats by injection of mycobacterium adjuvant. I. General clinic and pathological characteristics and some modifying factors. Arthritis Rheum 2:440–459

Perper RJ, Alvarez B, Colombo C, Schroder H (1971) The use of a standardized adjuvant arthritis assay to differentiate between anti-inflammatory and immunosuppressive agents. Proc Soc Exp Biol Med 137:506–512

Pircio AW, Fedele CT, Bierwagen ME (1975) A new method for the evaluation of analgesic activity using adjuvant-induced arthritis in the rat. Eur J Pharmacol 31:207–215

Roberts ED, Ramsey KF, Switzer WP, Layton JM (1968) Pathologic changes of porcine suppurative arthritis produced by *Streptococcus equisimilis*. Am J Vet Res 29:253–262

Roberts ED, Ramsey KF, Switzer WP, Layton JM (1969) Electron microscopy of porcine synovial membrane cell layer in *Streptococcus equisimilis* arthritis. J Comp Pathol 79:47–51

Rooks WH, Tomolonis AJ, Maloney PJ, Wallach MB, Schuler ME (1982) The analgesic and anti-inflammatory profile of (± − 5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2a]pyrrole-1-carboxylic acid (RS-37619). Agents Actions 12:684–690

Sandow J, Alpermann H, Metzger H, Vogel HG (1971) α-2-Glycoprotein levels in the experimental immunoarthritis of the rat. Naunyn-Schmiedeberg's Arch Pharmacol 269:483

Schorlemmer HU, Dickneite G (1992) Preclinical studies with 15-deoxyspergualin in various animal models for autoimmune diseases. Ann N Y Acad Sci 685:155–174

Schorlemmer HU, Kurrle R, Schleyerbach R, Bartlett RR (1999) Disease-modifying activity of malononinitrilamides, derivates of leflunomide's active metabolite, on models of rheumatoid arthritis. Inflamm Res 48(Suppl 2): S113–S114

Schulz LC, Drommer W, Seidler D, Ehard H, Mickwitz G, Hertrampf B, Böhm KH (1975a) Experimenteller Rotlauf bei verschiedenen Spezies als Ursache einer systemischen Bindegewebskrankheit. I. Systemische vaskuläre Prozesse bei der Organmanifestation. Beitr Path 154:1–20

Schulz LC, Drommer W, Seidler D, Ehard H, Leimbeck R, Weiss R (1975b) Experimenteller Rotlauf bei verschiedenen Spezies als Modell einer systemischen Bindegewebskrankheit. II. Chronische Phase mit besonderer Berücksichtigung der Polyarthritis. Beitr Pathol 154:27–51

Schulz LC, Ehard H, Hertrampf B, Drommer W, Seidler D, Böhm KH (1977) Hemostasis, fibrin incorporation and local mesenchymal reaction in erysipelothrix infection as a model for rheumatism research. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 215–237

Shi X-L, Wang L-P, Feng X, Fan D-D, Zang W-J, Wang B, Zhou J (2014) Inhibition of adjuvant-induced arthritis by nasal administration of novel synthetic peptides from heat shock protein 65. BMC Muskuloskelet Disord 15:252. doi: 10.1186/1471-2474-15-253

Shimizu G, Shichikawa K, Takiuchi K (1958) Studies on the etiology of rheumatic arthritis. III. Experimental production of arthritis in rabbits following focal infection of paranasal sinus with hemolytic streptococcus. Med J Osaka Univ 9:447–468

Snekhalatha U, Anburajan M, Venkatraman B, Menaka M (2013) Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model. Zeitschrift Rheumatol 72:375–388

Stein H, Yarom R, Levine S, Dishon T, Ginsburg I (1973) Chronic self-perpetuating arthritis induced in rabbits by a cell-free extract of group A streptococci. Proc Soc Exp Biol N Y 143:1106–1112

Tsurumi K, Kokuba S, Okada K, Yanagihara M, Fujimura H (1986) Pharmacological investigations of the new antiinflammatory agent 2-(10,11-dihydro-10-oxodibenzo[b, f]thiepin-2-yl) propionic acid. 4th communication: inhibitory effects on rat adjuvant arthritis. Arzneim-Forsch/Drug Res 36:1810–1817

Van Bilsen JHM, Wagenaar-Hilbers JPA, Grosfeld-Stulemeijer MCJT, van der Cammen MJF, van Dijk MEA, van Eden E, Wauben MHM (2004) Matrix metalloproteinases as targets for the immune system during experimental arthritis. J Immunol 172:5063–5068

Vingsbo C, Jonsson R, Holmdahl R (1995) Avridine-induced arthritis in rats: a T cell-dependent chronic disease influenced both by MHC genes and by non-MHC genes. Clin Exp Immunol 99:359–363

Vollmer S, Gemeinhardt I, Vater A, Schnorr B, Schnorr J, Voigt J, Ebert B (2014) In vivo therapy monitoring of experimental rheumatoid arthritis in rats using near-infrared fluorescence imaging. J Biomed Opt 19. doi: 10.1117/1.JBO.19.3.036011

Wei YH, Li Y, Qiang CJ (2004) Effects and mechanisms of FR167653, a dual inhibitor of interleukin-1 and tumor necrosis factor, on adjuvant arthritis in rats. Int Immunopharmacol 4:1625–1632

Walz DT, DiMartino MJ, Kuch JH, Zuccarello W (1969) Adjuvant-induced arthritis in rats – temporal relationship of drug effects on physiological, biochemical, and haematological parameters. Pharmacologist 11:266

Wang B (2014) Anti-arthritic effect of astragaloside IV and its molecular mechanism. Inflamm Cell Signal 1:e130

Weichman BM (1989) Rat adjuvant arthritis: a model of chronic inflammation. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 363–380

White TG, Jl P, Hargrave P (1975) Production of synovitis in rabbits by fractions of a cell-free extract of *Erysipelothrix rhusiopathiae*. Clin Immunol Immunopathol 3:531–540

Wilder RL, Calandra GB, Garvin AJ, Wright KD, Hansen CT (1982) Strain and sex variation in the susceptibility to streptococcal wall-induced polyarthritis in the rat. Arthritis Rheum 25:1064–1072

Wilder RL, Allen JB, Hansen C (1987) Thymus-dependent and -independent regulation of Ia antigen expression *in situ* by cells in the synovium of rats with streptococcal cell wall-induced arthritis. J Clin Invest 79:1160–1171

Yocum DE, Allen JB, Wahl SM, Calandra GB, Wilder RL (1986) Inhibition by cyclosporin A of streptococcal wall-induced arthritis and hepatic granulomas in rats. Arthritis Rheum 29:262–273

Zhang Z-C, Zhang S-J, Jin B, Wu Y, Yang X-F, Yu B, Xie Q-M (2014) Ciclamilast ameliorates adjuvant-induced arthritis in a rat model. BioMed Res Int. Article ID 786104 (in press)

Collagen Type II-Induced Arthritis in Rats

Purpose and Rationale

As reported by Trentham et al. (1977), intradermal injection of homologous or heterologous type II collagen in incomplete Freund's adjuvant results in an inflammatory polyarthritis in rats. The demonstration of antibodies to collagen in patients with rheumatic polyarthritis suggests that autoimmunity may contribute to the pathophysiology of synovitis and joint destruction. Because of the similarities of the symptoms in rats to human disease, the test is considered to be useful to detect anti-inflammatory and immunosuppressive properties of test compounds.

Procedure

Bovine type II collagen is prepared from nasal septum cartilage, which is cut into small fragments, frozen in liquid nitrogen, and pulverized in a freezer mill. Proteoglycans are extracted

overnight by stirring 25 g of pulverized cartilage in 1 l of 0.2 N NaOH. Following centrifugation at 20,000 g for 30 min, the residue is washed with 250 ml of absolute ethanol, the supernatant aspirated, and the residue vacuum dried. Hundred mg pepsin is added to 150 ml of 0.5 M acetic acid, after which 1.0 g of cartilage is added to reach a cartilage to pepsin ratio of 10:1 (w/w). The mixture is stirred 18 h at room temperature and centrifuged at 20,000 *g* for 1 h. Acid soluble collagen present in the supernatant is precipitated by adding NaCl to reach a final concentration of 0.9 M, followed by centrifugation at 20,000 g for 1 h. The precipitate from 1.0 g cartilage is dissolved in 100 ml 1.0 N NaCl/0.005 M Tris–HCl, pH 7.5, and stirred for 3 days. Then, the solution is dialyzed against 0.02 M Na_2HPO_4, pH 9.4, and the precipitate collected by centrifugation at 30,000 *g* for 1 h. The pellet is dissolved in 0.5 M acetic acid, dialyzed against 6 l of 0.01 M acetic acid, and lyophilized. All procedures, unless otherwise stated, are performed at 4 °C.

Test procedure. Collagen is dissolved in a concentration of 2.0 mg/ml in 0.1 M acetic acid overnight at 4 °C. This solution is added dropwise to an equal volume of chilled incomplete Freund's adjuvant. Six to 12 male Wistar rats with an initial weight of about 120 g are used for each group. On day 1, each rat receives a total of 0.5 mg collagen in 0.5 ml, equally divided, in five sites. All injections are intradermal, one at the base of each appendage and one in the nape of the neck. Seven days postimmunization, the animals receive identical booster injections. Control animals receive only the incomplete Freund's adjuvant diluted with 0.1 M acetic acid.

The volume of both hind paws is measured plethysmographically on day 20. To minimize the possibility of including animals with minimal transient disease, only animals with a paw volume of 1.8 ml or greater are used for further testing. From days 20–40, the animals receive the test compounds p.o. once a day. On day 41, the paw volumes are recorded again.

Evaluation

The paw volumes of treated animals are recorded plethysmographically. The increase versus day 20 is calculated. The increase is compared with that of controls or animals treated with a standard drug. Otherwise, arthritic scores can be determined. Nonsteroidal anti-inflammatory drugs such as indomethacin in a dose of 2 mg/kg p.o. or phenylbutazone in a dose of 150 mg/kg p.o., but not acetylsalicylic acid in a dose of 50 mg/kg p.o., have been found to be active. Likewise, corticosteroids and immunosuppressives, but not D-penicillamine, were active.

Critical Assessment of the Method

Nonsteroidal and steroidal anti-inflammatory compounds are detected by this method which, however, does not allow a separation between these two groups.

Modifications of the Method

From studies with a neutrophil elastase inhibitor, Janusz and Durham (1997) concluded that the destruction of the joints in rat collagen-induced arthritis is at least partially due to neutrophil elastase.

Romas et al. (2002) reported that osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis in Dark Agouti rats.

Studies in Mice

Hom et al. (1988), Takagishi et al. (1986, 1992), Cannon et al. (1990), Nemoto et al. (1992), and Carlson et al. (1992) described the effects of immunomodulating agents in collagen-induced arthritis in mice.

Wooley et al. (1993) investigated the anti-arthritic effect of recombinant human interleukin-1 receptor antagonist protein on type II collagen-induced arthritis and antigen-induced arthritis in mice.

Joosten et al. (1994) found an accelerated onset of collagen-induced arthritis in DBA_1 lac/J mice by remote inflammation.

Miesel and Haas (1993), Miesel et al. 1994a, b) studied the effects of an active center analogue of Cu_2Zn_2-superoxide dismutase in collagen type II-induced arthritis. Furthermore, the authors described a model potassium peroxochromate-induced inflammation in rats and mice. One to

3 μmol/kg K_3CrO_8 was administered by intraplantar application into the left hind paws of anesthetized rats or mice. Arthritis index was assessed by a score system, or the inflammatory response was quantified scintigraphically under a gamma camera by intravenous injection of 500 μCi $Na^{99m}TcO_4$.

Kumar et al. (1997) compared the cellular mechanisms involved in the control of collagen II-induced arthritis and experimental autoimmune encephalomyelitis in mice.

Ruchatz et al. (1998) studied the role of IL-15 in development of antigen-induced immunopathology in collagen-induced arthritis in DBA/1 mice. A soluble fragment of IL-15 receptor profoundly suppressed the symptoms of collagen-induced arthritis.

Joosten et al. (1999) immunized male DBA-1 mice with 100 μg bovine type II collagen in CFA enriched with *Mycobacterium tuberculosis* H37Ra (4 mg/ml) at the base of the tail. The mice were boosted i.p. with 100 μg collagen dissolved in saline. After disease onset on day 28, the mice were treated either with dimerically linked PEGylated soluble p55 TNFR1 receptor or with purified rabbit anti-murine IL-1α and anti IL-1β. IL-1$\alpha\beta$ blockade prevented cartilage and bone destruction, whereas TNF-α blockade only ameliorated joint inflammation.

Using a similar protocol, Plater-Zyberg et al. (2001) found a therapeutic effect of neutralizing endogenous IL-18 activity in the collagen-induced model of arthritis and Lubberts et al. (2004) after treatment with a neutralizing anti-murine interleukin-17 antibody.

Cuzzocrea et al. (2003) found a reduction in the evolution of murine type II collagen-induced arthritis by treatment with rosiglitazone, a ligand of PPARγ.

McIntyre et al. (2003) reported that a highly selective inhibitor of IκB kinase blocked both inflammation and destruction in collagen-induced arthritis in mice.

Chen et al. (2003) tested orally active inhibitors of TNF synthesis as anti-rheumatoid arthritis drugs using collagen-induced arthritis in male DBA/1 J mice.

Nakae et al. (2002, 2003) generated IL-17-deficient mice and found a suppression of collagen-induced arthritis.

Podolin et al. (2005) described attenuation of murine collagen-induced arthritis by a selective small-molecule inhibitor of IκB kinase 2, occurring via reduction of proinflammatory cytokines and antigen-induced T cell proliferation.

Kuno et al. (2006) reported anti-inflammatory activity of a non-nucleoside adenosine deaminase inhibitor in mice.

Hegen et al. (2008), Bevaart et al. (2010), Bolon et al. (2011), and Roy and Ghosh (2013) reviewed the utility of animal models in arthritis and their suitability for therapeutic target evaluation and correlation with clinical treatment of human rheumatoid arthritis. Many compounds have been evaluated in collagen-induced arthritis including inhibitors of the Bruton's tyrosine kinase (Liu et al. 2011), inhibitors of Sphingosine-1-phosphate (Fujii et al. 2012), and agonists of the nicotinic alpha7 receptor (Hu et al. 2014). Consistent with this finding, the role of the cholinergic pathway as an anti-inflammatory mechanism has been explored in this model (Levine et al. 2014). Furthermore, technological advances for imaging inflammation and monitoring therapeutic responses have been developed (Balducci et al. 2012; Sevilla et al. 2015), which may help progress the discovery and development of new drugs, where differentiation from drugs currently in clinical practice is mandated.

References and Further Reading

Anthony Balducci A, Helfer BM, Ahrens ET, O'Hanlon III CF, Wesa AK Visualizing arthritic inflammation and therapeutic response by fluorine-19 magnetic resonance imaging (^{19}F MRI). J Inflamm 9:24

Balducci A, Helfer BM, Ahrens ET, O'Hanlon CF, Wesa AK (2012) Visualizing arthritic inflammation and therapeutic response by fluorine-19 magnetic resonance imaging (19F MRI). J Inflamm 9:24. doi:10.1186/1476-9255-9-24

Bevaart L, Vervoordeldonk MJ, Tak PP (2010) Evaluation of therapeutic targets in animal

models arthritis. Arthritis Rheum 62:2192–2205

Bolon B, Stolina M, King C, Middleton S, Gasser J, Zack DU (2011) Rodent preclinical models for developing novel antiarthritic molecules: comparative biology and preferred methods for evaluating efficacy. J Biomed Biotechnol 2011. Article ID 569068, 21 pp. doi:10.1155/2011/569068

Cannon GW, McCall S, Cole BC, Griffiths MM, Radov LA, Ward JR (1990) Effects of indomethacin, cyclosporin, cyclophosphamide, and placebo on collagen-induced arthritis of mice. Agents Actions 29:315–323

Carlson RP, Baeder WL, Caccese RG, Warner LM, Sehgal SN (1992a) Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. Ann N Y Acad Sci 685:86–113

Chen JJ, Dewdney N, Lin X, Martin RL, Walker KAM, Huang J, Chu F, Eugui E, Mirkovich A, Kim Y, Sarma K, Arzeno H, van Wart HE (2003) Design and synthesis of orally active inhibitors of TNF synthesis as anti-rheumatoid arthritis drugs. Bioorg Med Chem Lett 13:3951–3954

Cuzzocrea S, Mazzon E, Dugo L, Patel NSA, Seraino I, di Paola R, Genovese T, Britti D, de Maio M, Caputi AP, Theimermann C (2003) Reduction in the evolution of murine type II collagen-induced arthritis by treatment with rosiglitazone, a ligand of the peroxisome proliferator-activated receptor γ. Arthritis Rheum 48:3544–3556

Fujii Y, Hirayama T, Ohtake H, Ono N, Inoue T, Sakurai T, Takayama T, Matsumoto K, Tsukahara N, Hidano S, Harima N, Nakazawa K, Igarashi Y, Goitsuka R (2012) Amelioration of collagen-induced arthritis by a novel SIP_1 antagonist with immunomodulatory activities. J Immunol 188:206–215

Hegen M, Keith JC Jr, Collins M, Nickerson-Nutter CL (2008) Utility of animal models for identification of potential therapeutics for rheumatoid arthritis. Ann Rheum Dis 67:1505–1515

Henderson H, Staines NA, Burrai I, Cox JH (1984) The anti-arthritic and immunosuppressive effects of cyclosporine on arthritis induced in the rat by type II collagen. Clin Exp Immunol 57:51–56

Hom JT, Butler LD, Riedl PE, Bendele AM (1988) The progression of the inflammation in established collagen-induced arthritis can be altered by treatments with immunological or pharmacological agents which inhibit T cell activities. Eur J Immunol 18:881–888

Hu Y, Liu R, Li J, Yue Y, Cheng W, Zhang P (2014) Attenuation of collagen-induced arthritis in rat by nicotinic alpha7 receptor partial agonist GTS-21. BioMed Res Int 2014. Article ID 325875, 9 pp. doi:10.1155/2014/325875

Janusz MJ, Durham SL (1997) Inhibition of cartilage degradation in rat collagen-induced arthritis but not adjuvant arthritis by the neutrophil elastase inhibitor MDL 101,146. Inflamm Res 46:503–508

Joosten LAB, Helsen MMA, Van den Berg WB (1994) Accelerated onset of collagen-induced arthritis by remote inflammation. Clin Exp Immunol 97:204–211

Joosten LAB, Helsen MMA, Saxne T, van de Loo FAJ, Heinegård D, van den Berg WB (1999) IL-1$\alpha\beta$ blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-α blockade only ameliorates joint inflammation. J Immunol 163:5049–5055

Kaibara N, Hotokebuchi T, Takagishi K, Katsuki I (1983) Paradoxical effects of cyclosporin A on collagen arthritis in rats. J Exp Med 158:2007–2015

Kumar V, Aziz F, Sercarz E, Miller A (1997) Regulatory T cells specific for the same framework 3 region of the Vβ8.2 chain are involved in the control of collagen II-induced arthritis and experimental autoimmune encephalomyelitis. J Exp Med 185:1725–1733

Kuno M, Seki N, Tsujimoto S, Nakanishi I, Kinoshita T, Nakamura K, Terasaka T, Nishio N, Sato A, Fujii T (2006) Anti-inflammatory activity of non-nucleoside adenosine deaminase inhibitor FR234938. Eur J Pharmacol 534:241–249

Levine YA, Koopman FA, Faltys M, Caravaca A, Bendele A, Zitnik R, Vervoordeldonk MJ, Tak PP (2014) Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. PLoS One. doi: 10.1371/journal.pone.0104530

Liu L, di Paolo J, Barbosa J, Rong H, Reif K, Wong H (2011) Antiarthritis effect of a novel Bruton's tyrosine kinase (BTK) inhibitor in rat collagen-induced arthritis and mechanism-based pharmacokinetic/pharmacodynamic modeling: relationships between inhibition of BTK phosphorylation and efficacy. J Pharmacol 338:154–163

Lubberts E, Koenders MI, Oppers-Walgreen B, van den Bersselaar L, Coenen-de Roo CJJ, Joosten LAB, van den Berg WB (2004) Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. Arthritis Rheum 50:650–659

McIntyre KW, Shuster DJ, Gillooly KM, Dambach DM, Pattoli MA, Lu P, Zhou XD, Zusi FC, Burke JR (2003) A highly selective inhibitor of IκB kinase, BMS-.345541, blocks both inflammation and destruction in collagen-induced arthritis in mice. Arthritis Rheum 48:2652–2659

Miesel R, Haas R (1993) Reactivity of an active center analog of Cu_2Zn_2-superoxide dismutase in murine model of acute and chronic inflammation. Inflammation 17:595–611

Miesel R, Dietrich A, Brandl B, Ulbrich N, Kurpisz M, Kröger H (1994a) Suppression of arthritis by an active center analogue of Cu_2Zn_2-superoxide dismutase. Rheumatol Int 14:119–126

Miesel R, Kröerg H, Ulbrich N, Kurpisz M (1994b) Arthritogenic reactivity of chromium (V). Z Rheumatol 53:59

Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, Sekikawa K, Asano M, Iwakura Y (2002) Antigen-specific T cell sensitization in impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 17:375–378

Nakae S, Nambu A, Sudo K, Iwakura Y (2003) Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J Immunol 171:6173–6177

Nemoto K, Mae T, Abe F, Takeuchi T (1992) Successful treatment with a novel immunosuppressive agent, deoxyspergualin, in type II collagen-induced arthritis in mice. Ann N Y Acad Sci 685:148–154

Phadke K, Carroll J, Nanda S (1982) Effects of various anti-inflammatory drugs on type II collagen-induced arthritis in rats. Clin Exp Immunol 47:579–586

Plater-Zyberg C, Joosten LAB, Helsen MMA, Sattonnet-Roche P, Siegfried C, Alouani S, van de Loo FAJ, Graber P, Aloni S, Cirillo R, Lubberts E, Dinarello CA, van den Berg WB, Chvatchko Y (2001) Therapeutic effect of neutralizing endogenous IL-18 activity in the collagen-induced model of arthritis. J Clin Invest 108:1825–1832

Podolin PL, Callahan JF, Bolognese BJ, Li YH, Carlson K, Davis TG, Mellor GF, Evans C, Roshak AK (2005) Attenuation of murine collagen-induced arthritis by a novel, potent, selective small molecule inhibitor of IκB kinase 2, TPCA-1 (2[(aminocarbonyl) amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide), occurs via reduction of proinflammatory cytokines and antigen-induced T cell proliferation. J Pharmacol Exp Ther 312:373–381

Romas E, Sims NA, Hards DK, Lindsay M, Quinn JWM, Ryan OFJ, Dunstan CR, Martin TJ, Gillespie MT (2002) Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. Am J Pathol 161:1419–1427

Roy T, Ghosh S (2013) Animal models of rheumatoid arthritis: correlation and usefulness with human rheumatoid arthritis. Indo Am J Pharm Res 3:6131–6142

Ruchatz H, Leung BP, Wi XQ, McInnes IB, Liew FY (1998) Soluble IL-15 receptor α-chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology. J Immunol 160:5654–5660

Probeert AW, Schrier DJ, Gilbertsen RB (1984) Effects of anti-arthritic compounds on type II collagen-induced arthritis in rats. Arch Int Pharmacodyn Ther 269:167–176

Sevilla RS, Cruz F, Chiu C-S, Xue D, Bettano KA, Zhu J, Chakravarthy K, Faltus R, Wang S, Vanko A, Robinson G, Zielstorff M, Miao J, Leccese E, Conway D, Moy LY, Dogdas B, Cicmil M, Zhang W (2015) Development and optimization of a high throughput micro-computed tomography imaging method incorporating a novel analysis technique to evaluate bone mineral density of arthritic joints in a rodent model of collagen induced arthritis. Bone 73:32–41

Takagishi K, Kaibara N, Hotokebuchi T, Arita C, Morinaga M, Arai K (1986) Effects of cyclosporin on collagen induced arthritis in mice. Ann Rheum Dis 45:339–344

Takagishi K, Yamamoto M, Miyahara H, Hotokebuchi T, Kaibara N (1992) Comparative study of effects of cyclosporins A and G on collagen arthritis in mice. Agents Actions 37:284–289

Tanaka K, Shimotori T, Makino S, Aikawa Y, Inaba T, Yoshida C, Takano S (1992) Pharmacological studies of the new anti-inflammatory agent 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one. 1st communication: anti-inflammatory, analgesic and other related properties. Arzneim Forsch/Drug Res 42:935–944

Trentham DE, Dynesius-Trentham RA (1989) Type II collagen-induced arthritis in the rat. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 395–413

Trentham DE, Townes AS, Kang AH (1977) Autoimmunity to type II collagen: an experimental model of arthritis. J Exp Med 146:857–868

Wooley PH, Whalen JD, Chapman DL, Berger AE, Richard KA, Aspar DG, Staite ND (1993) The effect of an interleukin-1 receptor antagonist protein on type II collagen-induced arthritis and antigen-induced arthritis in mice. Arthritis Rheum 36:1305–1314

Proteoglycan-Induced Progressive Polyarthritis in Mice

Purpose and Rationale

Glant et al. (1987, 1992), Mikecz et al. (1987, 1990), and Poole (1989) described a proteoglycan-induced progressive arthritis and spondylitis in BALB/c mice as an animal model displaying similarities to human rheumatoid arthritis and ankylosing spondylitis as indicated by clinical assessments, immunological parameters, and histopathological studies of diarthrodial joints and spine.

Procedure

High buoyant density cartilage proteoglycans are prepared from fetal and adult human, canine or bovine articular cartilages, as well as 1-week-old mouse epiphyseal cartilage. Fetal human articular cartilage proteoglycan digested with chondroitinase ABC (Hascall and Heinegård 1974) is used to induce arthritis in female BALB/c mice. The mice are sensitized by intraperitoneal injection of 100 μg of chondroitinase ABC-treated proteoglycan in 100 μl of phosphate-buffered saline, pH 7.2, and in Freund's complete adjuvant in a 1:1 emulsion. They are reinjected twice more with the antigen in incomplete Freund's adjuvant after 1 and 3 weeks. All BALB/c mice immunized with human articular cartilage proteoglycan develop arthritis in diarthrodial joints after the third antigen injection. Sera from mice with progressive polyarthritis are tested for antibodies to arthritogenic proteoglycans during weeks 12–18 of immunization. The limbs of all mice are examined daily to record clinical arthritic changes. Swelling and redness, as the first symptoms of arthritis, and the thickness (diameter) of the knee, ankle (intermalleolar diameter), wrist, and the dorsovolar thickness of the paw are recorded three times a week. The most objective joint diameter is the intermalleolar one. The animals are treated with test drug or vehicle for 12 weeks and serum samples taken by retro-orbital puncture for determination of antibodies to proteoglycans. Seven weeks later, the mice are sacrificed, and limbs, tails, and lumbar spine are

fixed, decalcified, and embedded in paraffin for histological examination.

Evaluation

Mean values of intermalleolar diameter and antibody titers of treated and non-treated animals are compared by nonparametric statistics.

Modifications of the Method

Stimpson and Schwab (1989) described a chronic remittent erosive arthritis in rats induced by bacterial peptidoglycan-polysaccharide structures.

Glant et al. (2011) extended this model to generate a model based on recombinant human glycan1 containing T cell epitopes suspected of being arthritogenic. Delemarre et al. (2014) explored the efficacy of autologous bone marrow transplantation in this model showing a stabilization of arthritis scores, and Swart et al. (2014) showed that mesenchymal stem therapy provided by either the intra-articular or intraperitoneal route may suppress proteoglycan-induced arthritis in a murine model.

References and Further Reading

Delemarre EM, Roord STA, van den Broek T, Zonneveld-Huijssoon E, de Jager W, Rozemuller H, Martens AC, Broere F, Wulffraat NM, Glant TT, Prakken BJ, van Wijk F (2014) Autologous stem cell transplantation in experimental arthritis by renewal and modulation of the Teff cell compartment. Arthritis Rheum 66:350–356

Glant TT, Mikecz K, Arzoumanian A, Poole AR (1987) Proteoglycan-induced arthritis in BALB/c mice: clinical features and histopathology. Arthritis Rheum 30:201–212

Glant TT, Mikecz K, Bartlett RR, Deák F, Thonar EJMA, Williams JM, Mattar T, Kuettner KE, Schleyerbach R (1992) Immunomodulation of proteoglycan-induced progressive polyarthritis by leflunomide. Immunopharmacology 23:105–116

Glant TT, Radacs M, Nagyeri G, Olasz K, Laszio A, Boldizsar F, Hegyi A, Finnegan A, Mikecz K (2011) Proteoglycan (PG)- induced arthritis (PGIA) and recombinant human PG-G1 domain-induced arthritis (GIA) in BALB/c mice resembling two subtypes of rheumatoid arthritis. Arthritis Rheum 63:1312–1321

Hascall VC, Heinegård D (1974) Aggregation of proteoglycans. I. The role of hyaluronic acid. J Biol Chem 249:4232–4241

Heinegård D (1972) Extraction, fractionation and characterization of proteoglycans from bovine tracheal cartilage. Biochim Biophys Acta 285:181–192

Mikecz K, Glant TT, Poole AR (1987) Immunity to cartilage proteoglycans in BALB/c mice with progressive polyarthritis and ankylosing spondylitis induced by injection of human cartilage proteoglycan. Arthritis Rheum 30:306–318

Mikecz K, Glant TT, Bukás E, Poole AR (1990) Proteoglycan-induced polyarthritis and spondylitis adoptively transferred to naive (nonimmunized) BALB/c mice. Arthritis Rheum 33:866–876

Poole AR (1989) Cartilage proteoglycan-induced arthritis: a combined model for rheumatoid arthritis and ankylosing spondylitis. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 441–447

Stimpson AS, Schwab JH (1989) Chronic remittent erosive arthritis induced by bacterial peptidoglycan-polysaccharide structures. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 381–394

Swart JF, de Roock S, Hofhuis FM, Rozemuller H, van den Broek T, Moerer P, Broere F, van Wijk F, Kuis W, Prakken BJ, Martens ACM, Wulffraat NM (2014) Mesenchymal stem cell therapy in proteoglycan induced arthritis. Ann Rheum Dis. doi: 10.1136/annrheumdis-2013-204147

Pristane-Induced Arthritis in Mice

Purpose and Rationale

The mineral oil 2,6,10,14-tetramethylpentadecane (known as **pristane**) induces a chronic inflammatory arthritis in **mice** after intraperitoneal injection (Potter and Wax 1981; Hopkins et al. 1984; Wooley et al. 1989; Chapdelaine et al. 1991;

Wooley and Whalen 1991; Levitt et al. 1992; Abe et al. 1995; Thompson et al. 1998; Wooley et al. 1998; Vigar et al. 2000). The immunological involvement in the pathogenesis of pristane-induced arthritis was studied by several authors (Bedwell et al. 1987; Thompson et al. 1990; Ghoraishian et al. 1993; Nishikaku et al. 1994; Vingsbo et al. 1996; Stasiuk et al. 1997; Morgan et al. 2004). Moreover, the genetic basis for the susceptibility to pristane-induced arthritis was studied (Lu et al. 2002; Olofsson et al. 2003; Brenner et al. 2005; Jensen et al. 2006). Not only in mice but also in **rats** arthritis could be induced by pristane injections (Vingsbo et al. 1996; Zheng et al. 2002, 2003; Webster et al. 2003; Holmberg et al. 2006).

Patten et al. (2004) characterized the model of pristine-induced arthritis (PIA) in mice by studying the response to antirheumatic agents, expression of joint cytokines, and immunopathology.

Procedure

Induction and Characterization of PIA

Male DBA/101aHsd mice were placed under isoflurane anesthesia and injected intraperitoneally with 0.5 ml of pristane (Sigma-Aldrich, Poole, UK), and an identical booster injection was given 7 weeks thereafter. The severity of arthritis was graded visually by assessing the level of swelling in each paw, including the tarsus (ankle) or carpus (wrist) joints. The following scoring system was used: 0.5 = swelling of toes only or very slight ankle/wrist swelling; 1 = slight swelling of paw; 2 = moderate swelling of paw; 3 = marked swelling of paw; and 4 = substantial swelling of paw. Thus, the maximum total score per animal was 16. All batches also contained animals that were not treated with pristane, and these served as comparators for all studies undertaken.

Mice were observed for paw or toe swelling in a time-course study lasting up to 180 days after the first pristane injection. After study termination, the initially swollen hind paws were obtained for histologic assessment and allocated to different study groups according to the duration of swelling. The remaining three paws of each animal were used in cytokine studies.

Drug Preparation and Administration Schedules

The effects of administration of established and novel antirheumatic compounds were assessed using a therapeutic dosing schedule. Separate batches of mice for each drug study were monitored weekly for the development of swollen paws from day 80 after the first injection of pristane. Mice were included in the drug studies only if they developed a score of ≥ 1 in a hind paw on two consecutive weekly observations between day 120 and day 134 after the first injection of pristane (n = 7–13 per treatment group). At study termination, paws were obtained for histologic and cytokine assessments, normally at 1 h after the final drug administration.

All orally administered treatments were undertaken by gavage. Prednisolone was suspended in 0.5 % methylcellulose and administered orally once daily at a dose of 2 mg/kg. Methotrexate was dissolved in physiologic saline and administered intraperitoneally three times per week at a dose of 9 mg/kg. Indomethacin and diclofenac were suspended in 1 % methylcellulose and given orally once daily at doses of 3 mg/kg and 2 mg/kg, respectively. Celecoxib was suspended in a solution of 66 % polyethylene glycol, 33 % water, and 1 % dimethyl sulfoxide and was administered orally twice daily at a dose of 30 mg/kg. Etanercept was dissolved in the supplied vehicle according to the instructions of the manufacturer and diluted using physiologic saline and was administered intraperitoneally three times per week at doses of 300 μg and 100 μg per mouse. Murine sTNFR, consisting of two murine p75 receptors fused to murine IgG2a, was dissolved in physiologic saline and administered intraperitoneally three times per week at doses of 300 μg and 100 μg per mouse. The selective p38 MAPK inhibitor SB242235 (synthesized at the US GSK Research Center) was suspended in 0.5 % tragacanth and 0.03 M hydrochloric acid and given orally twice daily at doses of 30 mg/kg and 15 mg/kg.

Joint Cytokine Messenger RNA (mRNA) and Protein Assays

The levels of mRNA and protein for the proinflammatory cytokines TNFα, IL-1β, and

IL-6 were measured in disaggregated joints by TaqMan real-time reverse transcription-polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISAs), respectively. At study termination and, in the drug studies, 1 h after the final drug treatment administration, the primary ankle joint was removed for histology, and the remaining paws were removed and snap-frozen in liquid nitrogen (six to eight mice per group). For cytokine assessment, the paw showing the highest score for swelling was selected with the proviso that it had also been swollen at the start of the drug study. If the remaining three paws exhibited no swelling at study termination, then the remaining ankle was selected for assay. Whole paws were frozen and pulverized using a mortar and pestle filled with liquid nitrogen.

For the mRNA studies, total RNA was isolated from homogenized paws using RNeasy Mini Kits (Qiagen, Crawley, UK). Samples were treated with 10 units of RNase-free DNase (Qiagen) for 15 min during the RNA isolation process. Reverse transcription of mRNA was carried out using TaqMan reverse transcription reagents in an MJ Research PTC-200 PCR Peltier Thermal Cycler. TaqMan probes and forward and reverse primers for the genes of interest (TNFα, IL-1β, and IL-6) and for housekeeping genes (GAPDH and cyclophilin) were designed with Primer Express TM software (PE Applied Biosystems). Cytokine mRNA expression levels were quantified by TaqMan real-time PCR using the ABI Prism 7900 Sequence Detector System (PE Applied Biosystems).

Measurement of Serum Antibody Levels

Blood was withdrawn from all mice before pristane injection and monthly thereafter. Levels of antibodies were determined by ELISA. Plates were coated with 100 µl of coating buffer (0.4 M phosphate buffer, pH 7.6) containing 5 µg of each antigen, at 4 °C overnight. The antigens assessed were bovine aggrecan, bovine biglycan, human endoplasmic reticulum molecular chaperone protein, bovine chondroitin sulfate A, bovine chondroitin sulfate B, bovine type I collagen, chick type II collagen, murine type II collagen peptide, bovine decorin, bovine double-stranded DNA, human fibronectin, lupine glucose-6-phosphate isomerase, mycobacterial 65-kDa heat shock protein, murine aggregated IgG, joint extract from normal mice, and joint extract from arthritic mice. Plates were washed three times with 0.05 % Tween 20 in PBS, and nonspecific binding was blocked by 5 % nonfat milk in PBS overnight at 4 °C. Serum samples from at least six individual mice per time point were used. Since 1:100 was the dilution determined to produce the optimal response to high-density proteoglycans, mouse serum diluted 1:100 in 5 % milk/PBS was added to each well and incubated overnight at 4 °C. Subsequently, the plates were washed six times with 0.05 % Tween 20 in PBS and incubated with alkaline phosphatase-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, Ala., USA) at 37 °C for 1 h. Plates were again washed six times and developed for 40 min in the dark, using *p*-nitrophenyl phosphate as a chromatogen substrate. The optical density was measured at 405 nm (OD_{405nm}) using an ultraviolet max spectrophotometer (Molecular Devices, Sunnyvale, Calif., USA). To ensure uniformity of the assay, negative control sera obtained prior to blood withdrawal and a standard mouse anti-type II collagen antiserum were titered on each plate. Antibody binding was expressed as the OD_{405nm} in units, blanked against control.

Isolation of Splenocytes and Cell Proliferation Assays

Spleens were excised and immediately immersed in PBS. Tissue was mechanically disrupted to release cells, which were suspended in 10 ml of sterile PBS and centrifuged for 10 min at 1,500 rpm. Prior to resuspension in medium, red blood cells were removed from the spleen preparations by adding distilled water for 10 s and then adding PBS. Spleen cells were then counted using a hemocytometer and washed and resuspended in RPMI at a final concentration of 2.5×10^6/ml.

Next, 100 µl of spleen cell aliquots (2.5×10^6/ml) was transferred to 96-well plates with 50 µg/ml of each antigen (aggrecan, biglycan, chondroitin sulfate A, chondroitin sulfate B, type I collagen, type II collagen, type II collagen peptide,

decorin, fibronectin, and heat shock protein; all were derived from the same species as described for the serum antibody studies) in complete RPMI 1640 medium. Cells were incubated for 72 h at 37 °C in the presence of antigen. Then 20 μl of MTT solution (a mitochondrial enzyme substrate) was added to each well (5 mg/ml). After a 6-h incubation, the culture supernatant was discarded, and 200 μl of 10 % sodium dodecyl sulfate solution was added to each well. After incubation at 37 °C overnight, the OD_{590nm} was read by microplate photospectrometer (Molecular Devices). The mean OD values were recorded for each cell sample as a measure of antigen stimulation. Antigen-specific responses were calculated as follows: (OD_{590nm} [stimulated culture]) – (OD_{590nm} [spontaneous proliferation culture]).

Histopathologic Evaluation

In all studies, the primary ankle joint that was swollen at the beginning of the time-course study or drug study was excised and fixed in 10 % neutral buffered formalin. The tissues were decalcified with formic acid and embedded in paraffin blocks. Sections (4–7 μm) were cut along a longitudinal axis, mounted, and stained with hematoxylin and eosin or toluidine blue, and representative slides for each animal were assessed. The following features were scored in six to ten animals per group: inflammatory exudate, neutrophil and mononuclear cell infiltration, bone resorption, and synovial hyperplasia. For drug studies, the effects of the agents on the pristane-induced pathologic condition were scored as follows: + = mild inhibition of pathologic features, ++ = moderate inhibition of pathologic features, and +++ = marked inhibition of pathologic features.

Evaluation

Graphic and tabular data are expressed as the mean ± SEM. Statistical significance was tested by application of the Kruskal–Wallis test for clinical scores and by analysis of variance followed by Dunnett's test for the cytokine mRNA and protein time-course results. Antibody and cell proliferation studies were analyzed using the least-squares significant difference post hoc test.

Modifications of the Method

Brenner et al. (2006) published thermal signature analysis as a novel method for evaluating inflammatory arthritis activity using rats with Freund's adjuvant-induced monoarthritis and pristane-induced arthritis. The thermal imaging system employs a platinum silicide 256 × 256 pixel detector array filtered to be sensitive to infrared radiation at a wavelength of 3–5 μm.

Lange et al. (2005) investigated the mode of action of methotrexate in different models for rheumatic arthritis, such as fibroblast-induced arthritis in SCID mice, collagen-induced arthritis and anti-collagen II antibody-induced arthritis in rats, and pristane-induced arthritis in DA rats, and models of multiple sclerosis, such as experimental autoimmune encephalomyelitis in (Balb/c × B10.Q) F1 and B10.Q mice.

Pristane induces lupus-like kidney and pulmonary disease in mice (Satoh et al. 1995; Richards et al. 1998; Lin et al. 2004; Chae et al. 2006).

De Franco et al. (2014) used the pristane-induced arthritis model to dissect genetic determinants for high inflammation susceptibility and demonstrate the involvement of loci interaction with the *Slc11a1* gene.

References and Further Reading

Abe C, Hirano S, Wakazono K, Mase T, Yamamoto R, Matsufuji M, Sakata N, Agata N, Iguchi H, Ishizuka M (1995) Effects of cytogenin on spontaneous arthritis in MRL/1 mice and on pristane-induced arthritis (PIA) in DBA/1J mice. Int J Tissue React 17:175–180

Bedwell AE, Elson CJ, Hinton CE (1987) The immunological involvement in the pathogenesis of pristane-induced arthritis. Scand J Immunol 25:393–398

Brenner M, Meng HC, Yarlett NC, Joe B, Griffiths MM, Remmers EF, Wilder RL, Gulko PS (2005) The non-MHC quantitative trait locus *Cia5* contains three major arthritis genes that differentially regulate disease severity, pannus formation, and joint damage in collagen- and pristane-induced arthritis. J Immunol 174:7894–7903

Brenner M, Braun C, Oster M, Gulka PS (2006) Thermal signature analysis as a novel method for evaluating inflammatory arthritis activity. Ann Rheum Dis 65:306–311

Chae BS, Park JS, Shin TY (2006) Endotoxin induces late increase un the production of pulmonary proinflammatory cytokines in murine lupus-like pristane-primed models. Arch Pharm Res 29:302–309

Chapdelaine JM, Whalen JD, Wooley PH (1991) Pristane-induced arthritis. II. Genetic regulation in F1 hybrid mice and cellular abnormalities following pristane injection. Autoimmunity 8:215–220

De Franco M, Peters LC, Correa MA, Galvan A, Canhamero T, Borrego A, Jensen A, Goncalves J, Cabrera WHK, Starobinas N, Ribeiro OG, Dragani T, Ibanez OM (2014) Pristane-induced arthritis loci interact with the Slc11a1 gene to determine susceptibility in mice selected for high inflammation. PLoS One. doi: 10.1371/journal.pone.0088302

Ghoraishian M, Elson CJ, Thompson SJ (1993) Comparison between the protective effects of mycobacterial 65-kD heat shock protein and ovomucoid in pristane-induced arthritis. relationship with agalactosyl IgG. Clin Exp Immunol 94:247–251

Holmberg J, Tuncel J, Yamada H, Lu S, Olofsson P, Holmdahl R (2006) Pristane, a non-antigenic adjuvant induces MHC class II-restricted, arthritogenic T cells in the rat. J Immunol 176:1172–1179

Hopkins SJ, Freemont AJ, Jayson MI (1984) Pristane-induced arthritis in BALB/c mice. I. Clinical and histological features of the arthropathy. Rheumatol Int 5:21–28

Jensen JR, Peters LC, Borrego A, Ribeiro OG, Cabrera WHK, Starobinas M, Siqueira M, Ibañez OCM, de Franco M (2006) Involvement of antibody trait loci in the susceptibility to pristane-induced arthritis in the mouse. Genes Immun 7:44–50

Lange F, Bajtner E, Rintisch C, Nandakumar KS, Sack U, Holmdahl R (2005) Methotrexate ameliorates T cell dependent autoimmune arthritis and encephalomyelitis but not antibody induced or fibroblast induced arthritis. Ann Rheum Dis 64:599–605

Levitt NG, Fernandez-Madrid F, Wooley PH (1992) Pristane induced arthritis in mice. IV. Immunotherapy with mononuclear antibodies directed against lymphocyte subsets. J Rheumatol 19:1342–1347

Lin L, Gerth AJ, Peng SL (2004) Susceptibility of mast cell-deficient W/Wv mice to pristane-induced experimental lupus nephritis. Immunol Lett 91:93–97

Lu S, Nordquist N, Holmberg J, Olofsson P, Pettersson U, Holmdahl R (2002) Both common and unique susceptibility genes in different rat strains with pristane-induced arthritis. Eur J Hum Genet 10:475–483

Morgan R, Wu B, Song Z, Wooley PH (2004) Immune reactivity to connective tissue antigens in pristane-induced arthritis. J Rheumatol 31:1497–1505

Nishikaku F, Aono S, Koga Y (1994) Protective effects of D-penicillamine and a thiazole derivative, SM-8849, on pristane-induced arthritis in mice. Int J Immunopharmacol 16:91–100

Olofsson P, Holmberg J, Pettersson U, Holmdahl R (2003) Identification and isolation of dominant susceptibility loci for pristane-induced arthritis. J Immunol 171:407–416

Patten C, Bush K, Rioja I, Morgan R, Wooley P, Trill J, Life P (2004) Characterization of pristine-induced arthritis, a murine model of chronic disease. Response to antirheumatic agents, expression of joint cytokines, and immunopathology. Arthritis Rheum 50:3334–3345

Potter M, Wax JS (1981) Genetics of susceptibility of pristine-induced plasmacytomas in BALB/sAn: reduced susceptibility in BALB/cJ with a brief description of pristine-induced arthritis. J Immunol 127:1591–1595

Richards HB, Satoh M, Shaw M, Libert C, Poli V, Reeves WH (1998) Interleukin 6 dependence of anti-DNA antibody production: evidence for two pathways of autoantibody formation in pristane-induced lupus. J Exp Med 188:985–990

Satoh M, Kumar A, Kanwar YS, Reeves WH (1995) Anti-nuclear antibody production and

immune-complex glomerulonephritis in BALB/c mice treated with pristane. Proc Natl Acad Sci U S A 92:10934–10938

Stasiuk LM, Ghoraishian M, Elson CJ, Thompson SJ (1997) Pristane-induced arthritis is $CD4^+$ T cell dependent. Immunology 90:81–86

Thompson SJ, Rook GA, Brealey TJ, van der Zee R, Elson CJ (1990) Autoimmune reactions to heat-shock proteins in pristane-induced arthritis. Eur J Immunol 20:2479–2484

Thompson SJ, Francis JN, Siew LK, Webb GR, Jenner PJ, Colston MJ, Elson CJ (1998) An immunodominant epitope from mycobacterial 65-kDa heat shock protein protects against pristane-induced arthritis. J Immunol 160:4628–4634

Vigar ND, Cabrera WH, Araujo LM, Ribeiro OG, Ogata TR, Siqueira M, Ibanez OM, de Franco M (2000) Pristane-induced arthritis in mice selected for maximal or minimal acute inflammatory reaction. Eur J Immunol 30:431–437

Vingsbo C, Sahlstrand P, Brun JG, Jonsson R, Saxne T, Holmdahl R (1996) Pristane-induced arthritis in rats: a new model for rheumatoid arthritis with a chronic disease course influenced by major histocompatibility complex and non-major histocompatibility complex genes. Am J Pathol 149:1675–1683

Webster L, Olofsson P, Ibrahim SM, Holmdahl R (2003) Chronicity of pristane-induced arthritis in rats is controlled by genes on chromosome 14. J Autoimmun 21:305–313

Wooley PH, Whalen JD (1991) Pristane-induced arthritis in mice. III Lymphocyte phenotypic and functional abnormalities precede the development of pristine-induced arthritis. Cell Immunol 138:251–259

Wooley PH, Seibold JR, Whalen JD, Chapedelaine JM (1989) Pristane-induced arthritis: the immunological and genetic features of an experimental murine model of autoimmune disease. Arthritis Rheum 32:1022–1030

Wooley PH, Sud S, Whalen JD, Nasser S (1998) Pristane-induced arthritis in mice. V. Susceptibility to pristine-induced arthritis is determined by the genetic regulation of the T cell repertoire. Arthritis Rheum 41:2022–2031

Zheng CL, Hossain MA, Kukita A, Ohki K, Satoh T, Kohashi O (2002) Complete Freund's adjuvant suppresses the development and progression of pristane-induced arthritis in rats. Clin Immunol 103:204–208

Zheng CL, Ohki K, Hossain MA, Kukita A, Satoh T, Kohashi O (2003) Complete Freund's adjuvant promotes the increase of IFN-γ and nitric oxide in suppressing chronic arthritis induced by pristane. Inflammation 27:247–255

Streptococcal Cell Wall-Induced Arthritis

Purpose and Rationale

Streptococcal cell wall (SCW)-induced arthritis is a chronic and erosive polyarthritis which may be induced in susceptible Lewis rats by a single injection of a sterile, aqueous suspension of SCW via the intraperitoneal route of administration (Cromartie et al. 1977).

The model has been used to study the efficacy of a number of experimental drugs which include the immunosuppressant cyclosporine A (Yocum et al. 1986); antibodies to IL-4, IL-10, interferon-γ, and monocyte chemotactic protein-1 (Schrier et al. 1998; Schimmer et al. 1998); the phosphodiesterase inhibitor rolipram (Laemont et al. 1999); the bisphosphonate clodronate (Richards et al. 2001); *N*-butyryl glucosamine (Wang et al. 2007); an inhibitor of the purinoreceptor $P2X_7$ (McInnes et al. 2014); and the TNF-a inhibitor etanercept (Chakravathy et al. 2014).

Procedure

Lewis rats, typically 120–150 g at the start of the study, receive an injection into the ankle joint of SCW (Lee Laboratories, Grayson, GA, USA). Susceptible animals can be identified by intra-articular injection of SCW (5 μg) into the ankle joint up to day 21 prior to any therapeutic intervention, which may reflect an acute phase of arthritis induction. The chronic, reactivation phase of the study, during which therapeutic intervention is typically investigated, is achieved by intravenous injection of SCW (100–200 μg). Studies normally run for 6–7 days post intravenous injection of SCW but may run for up to

30 days; animals are sacrificed prior to and after intravenous challenge for blood analysis and ankle joint assessment.

Evaluation

Disease severity is typically assessed using the following criteria:

1. A direct measurement of ankle swelling and mechanical hyperalgesia by von Frey threshold using nylon filaments
2. Assessment of histopathological measures which typically include synovitis, inflammation of synovial sub-lining, chondronecrosis, and subchondral bone resorption
3. Radiographical assessment of joint structure

It is also common practice to take blood samples for analysis of biomarkers and drug pharmacokinetics. Rioja et al. (2005) conducted an extensive analysis of the gene expression profile in response to SCW-induced arthritis.

Modification of the Method

Kuiper et al. (1998) used a single intravenous injection of SCw (25 μg) and assessed the effects of TNF-α and IL-1β blockade by administration of anti-cytokine antibodies 1 h prior to arthritis induction. Wang et al. (2007) induced arthritis by a single intraperitoneal injection of SCW (15 μg/g weight of rat) and studied the disease-modifying effects of *N*-butyryl glucosamine commencing the day after SCW injection.

References and Further Reading

Chakravathy K, Faltus R, Robinson G, Sevilla R, Shin J, Zielstorff M, Byford A, Leccese E, Caniga MJ, Hseih S, Zhang S, Chiu C-S, Zhang-Hoover J, Moy LY, McLeod RL, Stoffregen D, Zhang W, Murtaza A, Cicmil M (2014) Etanercept ameliorates inflammation and pain in a novel mono-arthritic multi-flare model of streptococcal cell wall induced arthritis. BMC Musculoskel Disord 15:409. doi: 11.1186/1471-2474-15-409

Cromartie WJ, Craddock JG, Schwab JH, Anderle SK, Yang C-H (1977) Arthritis in rats after systemic injection of streptococcal cells or cell walls. J Exp Med 146:1585–1602

Kuiper S, Joosten LAB, Bendele AM, Edwards CK III, Arntz OJ, Helsen MMA, van de Loo FAJ, van den Berg WB (1998) Different roles of tumor necrosis factor α and interleukin 1 in murine streptococcal cell wall arthritis. Cytokine 10:690–702

Laemont KD, Schaefer CJ, Juneau PL, Schrier DJ (1999) Effects of the phosphodiesterase inhibitor rolipram on streptococcal cell wall-induced arthritis in rats. Int J Immunopharmacol 21:711–725

McInnes IB, Cruwys S, Bowers K, Braddock M (2014) Targeting the $P2X_7$ receptor in rheumatoid arthritis: biological rationale for $P2X_7$ antagonism. Clin Exp Rheumatol 32:878–882

Richards PJ, Williams BD, Williams AS (2001) Suppression of chronic streptococcal cell wall-induced arthritis in Lewis rats by liposomal clodronate. Rheumatology 40:978–987

Rioja I, Clayton CL, Graham SJ, Life PF, Dickson MC (2005) Gene expression profiles in the rat streptococcal cell wall-induced arthritis model identified using microarray analysis. Arthritis Res Ther 7:R101–17

Schimmer RC, Schrier DJ, Flory CM, Laemont KD, Tung D, Metz AL, Friedl HP, Conroy MC, Warren JS, Beck B, Ward PA (1998) Streptococcal cell wall-induced arthritis: requirements for IL-4, IL-10, IFN-γ and monocyte chemoattractant protein-1. J Immunol 160:1466–1471

Schrier DJ, Schimmer RC, Flory CM, Tung DK-L, Ward PA (1998) Role of chemokines and cytokines in a reactivation model of arthritis induced by injection with streptococcal cell walls. J Leukoc Biol 63:359–363

Van den Broek MF, van den Berg WB, van de Putte LBA, Severijnen AJ (1988) Streptococcal cell wall-induced arthritis and flare-up reaction in mice induced by homologous or heterologous cell walls. Am J Pathol 133:139–149

Van den Broek MF, de Heer E, van Bruggen MCJ, de Roo G, Kleiverda K, Eulderink F, van den Berg WB (1992) Immunomodulation of

streptococcal cell wall-induced arthritis. Identification of inflammatory cells and regulatory T cell subsets by mercuric chloride and in vivo CD8 depletion. Eur J Immunol 22:3091–3095

Wang SX, Cherian A, Dumitriu M, Grynpas MD, Carran J, Wainman D, Anastassiades T (2007) Disease modifying effects of *N*-butyryl glucosamine in a streptococcal cell wall induced arthritis model in rats. J Rheumatol 34:712–720

Yocum DE, Allen JB, Wahl SM, Calandra GB, Wilder RL (1986) Inhibition by cyclosporin A of streptococcal cell wall-induced arthritis and hepatic granulomas in rats. Arthritis Rheum 29:262–273

Experimental Autoimmune Thyroiditis

Purpose and Rationale

Immunization of rats or mice with porcine thyroglobulin results in thyroiditis (Vladutiu and Rose 1971; Vladutiu 1983; McGregor et al. 1983; Hassman et al. 1985; Salamero et al. 1987; Fournier et al. 1990).

Procedure

Crude porcine thyroglobulin (PTg) solution is emulsified in complete Freund's adjuvant in a 1:1 ratio. Female mice (6–8 weeks old) are primed with 50 μg PTg given s.c. into four or five sites of injection and are boosted 14 days later with the same dose of PTg (s.c.) emulsified in incomplete Freund's adjutant. The test compounds are administered from day 0 (at priming) until day 21. Mice are bled on day 21 and on day 28 after priming. The sera are tested for the levels of anti-PTg antibodies using an enzyme-linked immunosorbent assay (ELISA). On day 28, the animals are sacrificed and the thyroid glands prepared. Five-micrometer-thick sections are stained with Masson-Goldner's trichrome solution.

Evaluation

The histological severity of experimental autoimmune thyroiditis is graded as a function of mononuclear cell thyroid infiltration indices:

1. Interstitial accumulation of inflammatory cells distributed between two or more follicles
2. One or two foci of inflammatory cells reaching at least the size of one follicle
3. 10–40 % of the thyroid replaced by inflammatory cells
4. More than 40 % of the thyroid replaced by inflammatory cells

Mean values of treated animals are compared with controls.

Modifications of the Method

Castagliola et al. (1994) induced autoimmune thyroid disease in BALB/c mice by immunizing with the extracellular domain of the human TSH receptor expressed as a maltose-binding protein fusion in bacteria. This type of thyroiditis could be transferred to naive BALB/c and NOD mice (Castagliola et al. 1996).

Green et al. (1995) described a spontaneous model of autoimmune thyroiditis in MRL-lpr/lpr mice.

Furthermore, Green et al. (1996) induced thyroiditis in Lewis rats by immunization with thyroid extract and thyroglobulin. A reduction of the gap junction proteins connexin 43, connexin 32, and connexin 26 was found in diseased thyroid tissue.

Wang et al. (2014) showed that overexpression of the human BH3 interacting-domain death agonist (BID) in the thyroids of transgenic mice may increase their sensitivity to iodine-induced autoimmune thyroiditis, noting that BID expression alone is not sufficient to induce thyroiditis.

References and Further Reading

Castagliola S, Many MC, Stalmans-Falys M, Tonacchera M, Vassart G, Ludgate M (1994) Recombinant thyrotropin receptor and the induction of autoimmune thyroid disease in BALB/c mice: a new animal model. Endocrinology 135:2150–2159

Castagliola S, Many MC, Stalmans-Falys M, Vassart G, Ludgate M (1996) Transfer of thyroiditis, with syngeneic spleen cells sensitized with the human thyrotropin receptor, to naive

BALB/c and NOD mice. Endocrinology 137:4637–4643
Fang Y, Zhao L, Parker CA, Yan F, Zhang C (2010) Modulation of apoptosis: new opportunities for drug discovery to treat autoimmune thyroiditis. Recent Pat Inflamm Allergy Drug Discov 4:255–260
Fournier C, Gepner P, Saouk M, Charreire J (1990) In vivo beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D_3 on the induction of experimental autoimmune thyroiditis. Clin Immunol Immunopathol 54:53–63
Green LM, LaBue M, Lazarus JP, Colburn KK (1995) Characterization of autoimmune thyroiditis in MRL-lpr/lpr mice. Lupus 4:187–196
Green LM, LaBue M, Lazarus JP, Jennings JC (1996) Reduced cell-cell communication in experimentally induced autoimmune thyroid disease. Endocrinology 137:2823–2832
Hassman RA, Dieguez C, Rennie DP, Weetman AP, Hall R, McGregor AM (1985) The influence of cyclosporin A on the induction of experimental autoimmune thyroid disease in the PVG/c rat. Clin Exp Immunol 59:10–16
Iddah MA, Macharia BN (2013) Autoimmune thyroid disorders. ISRN Endocrinol 2013. Article ID 509764, 9 pp. doi:10.1155/2013/509764
McGregor AM, Rennie PD, Weetman AP, Hassman RA, Foord SM, Dieguez C, Hall R (1983) The influence of cyclosporin A on experimental autoimmune thyroid disease in the rat. Life Sci 32:97–108
Luo Y, Kawashima A, Ishido Y, Yoshihara A, Oda K, Hiroi N, Ito T, Ishii N, Suzuki K (2014) Iodine excess as an environmental risk factor for autoimmune thyroid disease. Int J Mol Sci 15:12895–12912
Mori K, Yoshida K, Ishi K, Moroshi K, Nakagawa Y, Hoshikawa S, Ozaki H, Takahashi Y, Ito S (2011) Experimental autoimmune thyroiditis in human parvovirus B10 transgenic mice. Autoimmunity 44:483–489
Penhale WJ, Farmer A, Irvine WJ (1975) Thyroiditis in T cell-depleted rats: influence of strain, radiation dose, adjuvants and antilymphocyte serum. Clin Exp Immunol 21:362–375
Salamero J, Remy JJ, Michel-Béchet M, Chareire J (1987) Experimental autoimmune thyroiditis induced by a 5–10 kDa tryptic fragment from porcine thyroglobulin. Eur J Immunol 17:843–848
Tamura K, Woo J, Murase N, Nalesnik M, Thomson AW (1993) Inhibitory effect of FK 506 on autoimmune thyroid disease in the PVG rat. Ann N Y Acad Sci 696:257–262
Vladutiu AO (1983) Effect of cyclosporine on experimental autoimmune thyroiditis in mice. Transplantation 35:518–520
Vladutiu AO, Rose NR (1971) Autoimmune murine thyroiditis relation to histocompatibility (H-2) type. Science 174:1137–1139
Wang SH, Fan Y, Baker JR Jr (2014) Overexpression of BID in thyroids of transgenic mice increases sensitivity to iodine-induce autoimmune thyroiditis. J Transl Med 12:180

Coxsackievirus B3-Induced Myocarditis

Purpose and Rationale

The effects of immunosuppressant drugs can be studied in the murine model of coxsackievirus B3 myocarditis.

Procedure

Three-week-old male BALB/c mice are kept for 7 days before the experiment in a single, self-contained animal isolation unit to exclude pre-diseased animals. They are maintained in disposable, filter-topped cages and handled with gloves by gowned and masked personnel. The intraperitoneal route is used for injection of virus in a 0.5 ml volume.

The CVB3 virus strain is grown on either Hep-2 or VERO cells, aliquoted, and maintained at −70 °C until use. At the time of infection, seed virus is grown on either VERO or LLC-MK-2 cells with Dulbecco's modified Eagle medium, 12 % fetal calf serum, and gentamicin. Virus is harvested and adjusted to an inoculum of 1.75×10^7 plaque-forming units/0.5 ml RPM-1640. The test drugs are given subcutaneously daily for 8 days. On day 8, the animals are sacrificed, the hearts rapidly removed, and

divided into two equal cross sections. The basal portion is snap frozen for isolation of virus and determination of drug level. The apical portion is fixed in 10 % formalin, dehydrated, and embedded in paraffin. Five-mm sections are stained with hematoxylin–eosin and Masson's trichrome stains. The bases of the individual hearts are minced with a sterile scalpel, suspended in 1 ml RPMI-1640, and homogenized in a glass tissue grinder. The suspension is centrifuged at 8,000 *g* for 10 min at 4 °C. Supernatants are harvested and frozen at −70 °C until assay. Serial tenfold dilutions of heart homogenates in minimum essential medium are layered on confluent, 72-h-old VERO cells that had been grown in 96-well microtiter plates. Monolayers are checked daily for 7 days for presence or absence of virus and rate of cell destruction.

Evaluation

The slides are examined by two observers blinded to the slide code, and inflammation and necrosis are quantitated.

Modifications of the Method

Lane et al. (1991) showed that lipopolysaccharides promote CB3-induced myocarditis in otherwise resistant B10. A mice.

Beisel et al. (1991) identified a putative shared epitope between coxsackievirus B4 and mouse alpha cardiac myosin heavy chain.

Gauntt et al. (1993) found that epitopes shared between coxsackievirus B3 and normal heart tissue contribute to CVB3-induced myocarditis in mice.

Xu et al. (2004) used the murine model to deliver a chitosan–DNA vaccine and showed protection against acute CVB3 challenge. Park et al. (2009) and Yue et al. (2009) further explored approaches supportive of potential immunotherapeutics in this model using pancreatitis as an additional endpoint (Park et al. 2009). The model has also been used to investigate the innate immune response as a predictor for the progression of cardiovascular disease and heart failure in male mice (Onyimba et al. 2011) and to better understand the efficacy of further immunotherapeutic approaches where oral administration of interferon-α2b-transformed *Bifidobacterium longum* was shown to protect animals from CVB3-induced myocarditis (Yu et al. 2011).

A number of other agents have been tested in this model and include galectin-9 which ameliorated CVB3-induced myocarditis (Lv et al. 2011), IL-17 which was found to be protective (Xie et al. 2012), and the micro-RNA miR-21 which alleviated CVB3-induced myocarditis (He et al. 2013). A comparison of the effects of ivabradine and carvedilol showed an expected effect on heart rate reduction and a potential anti-inflammatory effect in the CVB3-induced myocarditis model.

Instead of coxsackievirus B3, Monrad et al. (1986) used encephalomyocarditis virus to induce experimental myocarditis in mice.

References and Further Reading

Beisel KW, Srinivasappa J, Prabhakar BS (1991) Identification of a putative shared epitope between Coxsackie virus B4 and alpha cardiac myosin heavy chain. Clin Exp Immunol 86:49–55

Chai D, Yue Y, Xu Y, Dong C, Xiong S (2014) Mucosal co-immunisation with AIM2 enhances protective SIgA response and increases prophylactic efficacy of chitosan-DNA vaccine against coxsackievirus B3-induced myocarditis. Hum Vaccin Ther 10:1284–1294

Estrin M, Huber SA (1987) Coxsackie virus B3-induced myocarditis. Autoimmunity is $L3T4^+$ T helper cell and IL-2 independent in Balb/c mice. Am J Pathol 127:337–341

Estrin M, Smith C, Huber S (1986) Coxsackie virus B-3 myocarditis. T-cell autoimmunity to heart antigens is resistant to cyclosporin-A treatment. Am J Pathol 125:244–251

Estrin M, Herzum M, Buie C, Huber SA (1987) Immunosuppressives in murine myocarditis. Eur Heart J 8(Suppl J):259–262

Fairweather D, Frisancho-Kiss S, Njoku DB, Nyland JF, Kaya Z, Yusung SA, Davis SE, Frisancho JA, Barrett MA, Rose NR (2006) Complement receptor 1 and 2 deficiency increases Coxsackievirus B3-induced myocarditis, dilated cardiomyopathy and heart failure

by increasing macrophages, IL-1b, and immune complex deposition in the heart. J Immunol 176:3516–3524

Gauntt CJ, Higdon HL, Arizpe HM, Tamayo MR, Crawley R, Henkel RD, Pereira MEA, Tracy SM, Cunningham MW (1993) Epitopes shared between Coxsackievirus B3 (CVB3) and normal heart tissue contribute to CVB3-induced murine myocarditis. Clin Immunol Immunopathol 68:129–134

He J, Yue Y, Dong C, Xiong S (2013) MiR-21 confers resistance against CVB3-induced myocarditis by inhibiting PDCD4-mediated apoptosis. Clin Invest Med 36:E103–E111

Huber SA, Lodge PA (1984) Coxsackie virus B-3 myocarditis in Balb/c mice. Evidence for autoimmunity to myocyte antigens. Am J Pathol 116:21–29

Huber SA, Lodge PA (1986) Coxsackie virus B-3 myocarditis. Identification of different pathogenic mechanisms in DBA/2 and Balb/c mice. Am J Pathol 122:284–291

Lane JR, Neumann DA, Lafond-Walker A, Herskowitz A, Rose NR (1991) LPS promotes CB3-induced myocarditis in B10. A mice. Cell Immunol 136:219–233

Lv K, Xu W, Wang C, Niki T, Hirashima M, Xiong S (2011) Galectin-9 administration ameliorates CVB3 induced myocarditis by promoting the proliferation of regulatory T cells and alternatively activated Th2 cells. Clin Immunol 140:92–101

Monrad ES, Matsumori A, Murphy JC, Fox JG, Crumpacker CS, Abelmann WH (1986) Therapy with cyclosporine in experimental murine myocarditis with encephalomyocarditis virus. Circulation 7:1058–1064

O'Connell JB, Reap EA, Robinson JA (1986) The effects of cyclosporine on acute murine Coxsackie B-3 myocarditis. Circulation 73:353–359

Onyimba JA, Coronado MJ, Garton AE, Kim JB, Bucek A, Bedja D, Gabrielson KL, Guilarte TR, Fairweather D (2011) The innate immune response to coxsackievirus B3 predicts progression to cardiovascular disease and heart failure in male mice. Biol Sex Differ. 2011 Feb 21;2:2. doi:10.1186/2042-6410-2-2

Park J-H, Kim D-S, Cho Y-J, Kim Y-J, Jeong S-Y, Lee S-M, Cho S-J, Yun C-W, Jo I, Nam J-H (2009) Attenuation of coxsackievirus B3 by VP2 mutation and its application as a vaccine against virus-induced myocarditis and pancreatitis. Vaccine 27:1974–1983

Rose NR, Herskowitz A, Neumann DA (1993) Autoimmunity in myocarditis: models and mechanisms. Clin Immunol Immunopathol 68:95–99

Xie Y, Chen R, Zhang X, Yu Y, Yang Y, Zou Y, Ge J, Chen H (2012) Blockade of interleukin-17A protects against Coxsackievirus B3-induced myocarditis by increasing COX-2/PGE2 production in the heart. FEMS Immunol Med Microbiol 64:343–351

Xu W, Shen Y, Jiang Z, Wang Y, Chu Y, Xiong S (2004) Intranasal delivery of chitosan-DNA vaccine generates mucosal SIgA and anti-CVB3 protection. Vaccine 22:3603–3612

Yu Z, Huang Z, Shao C, Huang Y, Zhang F, Yang J, Deng L, Zeng Z, Deng Q, Zeng W (2011) Oral administration of interferon-a-2b-transformed *Bifidobacterium longum* protects BALB/c mice against Coxsackievirus B3-induced myocarditis. Virol J 8:525

Yue Y, Gui J, Xu W, Xiong S (2011) Gene therapy with CCL2 (MCP-1) mutant protects CVB3-induced myocarditis by compromising Th1 polarization. Mol Immunol 48:706–713

Yue Y, Xu W, Hu L, Jiang Z, Xiong S (2009) Enhanced resistance to coxsackievirus B3-induced myocarditis by intranasal co-immunization of lymphotactin gene encapsulated in chitosan particle. Vaccine 386:438–447

Yue-Chun TZ, Na-Dan Z, Li-Sha G, Qin L, Xue-Qiang G, Jia-Feng L (2012) Comparison of effects of Ivabradine versus Carvedilol in murine model with the Coxsackie virus B3-induced viral myocarditis. PLoS One 7: e39394

Porcine Cardiac Myosin-Induced Autoimmune Myocarditis in Rats

Purpose and Rationale

Pummerer et al. (1991), Inomata et al. (1995), Suzuki (1995), and Dimitrijevic et al. (1998)

described autoimmune myocarditis in rats induced by porcine cardiac myosin.

Procedure

Male Sprague–Dawley or Lewis rats at the age of 8–10 weeks are immunized with porcine cardiac myosin either purchased from Sigma (St. Louis, MO, USA) or prepared from the ventricular muscle of porcine hearts according to Murakami et al. (1976). The cardiac myosin fraction is dissolved in phosphate buffer at a concentration of 10 mg/ml. The antigen solution is emulsified with equal volume of complete Freund's adjuvant supplemented with heat-killed mycobacterium tuberculosis. Rats are injected subcutaneously into the foot pad with an immunizing dose of 5 mg of antigen in complete Freund's adjuvant/kg of body weight. Rats are injected intraperitoneally with test compounds either from day 0 to 6 (early treatment group) or from day 14 to 20 (late treatment group).

Immunized rats are sacrificed on days 8, 16, 21, and 34, respectively. Disease course and severity are analyzed by macroscopic findings of the hearts and heart weight/bodyweight ratio as well as by histological and immunohistochemical analysis. Macroscopic findings are scored as follows: 0, normal finding; 1, presence of focal discolored area on the surface; and 2, presence of diffuse discolored areas (Kodama et al. 1995).

The hearts are removed and weighted immediately after the rats are sacrificed, fixed in 10 % buffered formalin, and embedded in paraffin. Serial section (5 μm in thickness) is stained with hematoxylin–eosin. The severity of myocarditis is determined according to the following scoring system: 0, no inflammation; 1, histological cross section infiltrated up to 5 %; 2, 5–10 % infiltrates/section; 3, 10–20 % infiltrates/section; greater than 20 % infiltrates/section.

For immunohistochemical staining, heart samples are embedded in OCT compound (Miles, Elkhart, IN) and rapidly frozen. Cryostat sections are cut sequentially at 7 μm in thickness, mounted on glass slides, and prepared for immunoperoxidase staining. Sections are fixed in cold acetone for 10 min and extensively washed in 0.1 M Tris buffer solution, pH 7.6. Murine monoclonal antibodies specific for different rat molecules are added at appropriate concentrations. After incubation at 4 °C overnight and further buffer washes, the sections are incubated with peroxidase-conjugated anti-mouse immunoglobulins for 60 min. Peroxidase reaction is visualized with 0.05 % diaminobenzidine in 0.01 % H_2O_2 for 7–8 min. The color development is stopped by washing slides in running water. All samples are lightly counterstained with hematoxylin, mounted in gelatin/glycerol medium, and assessed by light microscopy.

Evaluation

Macroscopic and microscopic scores are expressed as mean values. Body weights, heart weights, and heart weight/body weight ratio are expressed as mean ± SD. Student's *t*-test for paired samples is used for comparison data within groups in reference to time, while two-sample *t*-test is used for comparison data between groups.

Modifications of the Method

Koyama et al. (1995) immunized Lewis rats with human cardiac myosin suspended in complete Freund's adjuvant and induced severe active myocarditis with acute and chronic heart failure. The baseline left ventricular pressure was significantly lower in the chronic phase group, and peak dP/dt was significantly lower in both the acute phase group and the chronic phase group than in the respective controls. The animal model was recommended to study both acute heart failure related to acute myocarditis and chronic heart failure due to diffuse myocardial fibrosis.

Neu et al. (1990, 1991; Neu and Ploier 1991; Penninger et al. 1993) induced severe autoimmune myocarditis in some mouse strains by immunization with cardiac myosin in complete Freund's adjuvant.

Wahed et al. (2005) used the method of immunization with porcine cardiac myosin to test the effects of eplerenone, a selective aldosterone blocker, on the progression of left ventricular dysfunction and remodeling in rats with dilated cardiomyopathy.

References and Further Reading

Cammock CE, Halnon NJ, Skoczylas J, Blanchard J, Bohm R, Miller CJ, Lai C, Krogstad PA (2013) Myocarditis, disseminated infection, and early viral persistence following experimental coxsackievirus B infection of cynomolgus monkeys. PLoS One 8:e74569

Dimitrijevic M, Milenkovic M, Milosavljevic P, Stojic-Vukanic Z, Colic M, Bartlett R (1998) Beneficial effects of leflunomide on cardiac myosin-induced experimental autoimmune myocarditis in rats. Int J Immunother 14:9–21

Gong X, Han B, Zou Y, Wang J, Yang W (2014) Attenuation of experimental autoimmune myocarditis by si-RNA mediated CD40 silencing. Int Heart J 55:539–545

Hirano E, Shimada K, Komiyama T, Fulita M, Kishimoto C (2013) Erythromycin treatment suppresses myocardial injury in autoimmune myocarditis in rats via suppression of superoxide production. Int J Cardiol 167:2228–2233

Hoetzenecker K, Zimmermann M, Hoetzenecker W, Schwieger T, Kollmann D, Mildnerr M, Hegedus B, Mitterbauer A, Hacker S, Birner P, Gabriel C, Gyongyosi M, Blyszczuk P, Eriksson U, Ankersmit HJ (2013) Mononuclear cell secretome protects from experimental autoimmune myocarditis. Eur Heart J. doi: 10.1093/eurheartj/ehs459

Inomata T, Hanawa H, Miyanishi T, Yajima E, Nakayama S, Maita T, Kodama M, Izumi T, Shibata A, Abo T (1995) Localization of porcine cardiac myosin epitopes that induce experimental autoimmune myocarditis. Circ Res 76:726–733

Kodama M, Zhang S, Hanawa H, Saeki M, Inomata T, Suzuki K, Koyama S, Shibata A (1995) Effects of 15-deoxyspergualin on experimental autoimmune giant cell myocarditis of the rat. Circulation 91:1116–1122

Koyama S, Kodama M, Izumi T, Shibata A (1995) Experimental rat model representing both acute and chronic failure related to autoimmune myocarditis. Cardiovasc Drugs Ther 9:701–707

Milenkovic M, Arsenovic-Ranan N, Vucicevic D, Bufan B, Stojic-Vukanic Z (2007) Fusidin ameliorates experimental autoimmune myocarditis in rats by inhibiting TNF-α production. Pharmazie 62:445–448

Murakami U, Uchida K, Hiratsuka T (1976) Cardiac myosin from pig heart ventricle: purification and enzymatic properties. J Biochem 80:611

Myers JM, Cunningham MW, Fairweather D, Huber SA (2013) Autoimmune myocarditis, valvulitis and cardiomyopathy. Curr Protoc Immunol. doi: 10.1002/0471142735.im1514s101

Neu N, Ploier B (1991) Experimentally induced autoimmune myocarditis: production of heart myosin-specific antibodies. Autoimmunity 8:317–322

Neu N, Ploier B, Ofner C (1990) Cardiac myosin-induced myocarditis. Heart autoantibodies are not involved in the induction of the disease. J Immunol 145:4094–4100

Neu N, Klieber R, Frühwirth M, Berger P (1991) Cardiac myosin-induced myocarditis as a model of postinfectious autoimmunity. Eur Heart J Suppl D:117–120

Oh H, Ahn M, Matsumoto Y, Shiu T (2013) Alternatively activated M2 macrophages increase in early stages of experimental autoimmune myocarditis in Lewis rats. Korean J Vet Res 53:225–230

Pando R, Barshack I, Raz A, Luboshits G, Haklai R, Maysel-Auslender S, Kloog Y, Keren G, George J (2010) The Ras antagonist farnesylthiosalicylic acid ameliorates experimental myocarditis in the rat. Cardiovasc Pathol 19:94–101

Penninger JM, Neu N, Timms E, Wallace VA, Koh DR, Kishihara K, Pummerer C, Mak TW (1993) Induction of experimental autoimmune myocarditis in mice lacking CD3 or CD8 molecules. J Exp Med 178:1837–1842

Pummerer C, Berger P, Frühwirth M, Ofner C, Neu N (1991) Cellular infiltrate, major histocompatibility antigen expression and immunopathogenic mechanisms in cardiac myosin-induced myocarditis. Lab Invest 65:538

Sukumaran V, Veeraveedu PT, Gurusamy N, Yamaguchi K, Lakshmanan AP, Ma M, Suzuki K, Kodoma M, Watanabe K (2011)

Cardioprotective effects of Telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/Mas receptor axis. Int J Biol Sci 7:1077–1092

Suzuki J-I, Ogawa M, Muto S, Itai A, Isobe M (2008) A specific inhibitor of plasminogen activator inhibitor-1 suppresses rat autoimmune myocarditis. Expert Opin Ther Targets 12:1313–1320

Suzuki K (1995) A histological study on experimental autoimmune myocarditis with special reference to initiation of the disease and cardiac dendritic cells. Virchows Arch 426:493–500

Veia A, Cavallino C, Bacchini S, Pastore F, Lupi A, Rognoni A, Rametta F, Bongo AS (2014) Idiopathic giant cell myocarditis: state of the art. World J Cardiovasc Dis 4:316–324

Wahed MII, Watanabe K, Ma M, Yamaguchi K, Takahashi T, Tachikawa H, Kodame M, Aizawa Y (2005) Effects of eplerenone, a selective aldosterone blocker, on the progression of left ventricular dysfunction and remodeling in rats with dilated cardiomyopathy. Pharmacology 73:81–88

Experimental Allergic Encephalomyelitis

Purpose and Rationale

Experimental allergic encephalomyelitis was first produced in laboratory animals by Rivers et al. in 1933. This pathological model is an immunologic disease arising from a delayed hypersensitivity reaction to nervous tissue. In many respects, the model resembles autoimmune diseases, especially demyelinating diseases, in man (Constantinescu et al. 2011), and the utility of animal models as for drug discovery and development for neurological diseases especially multiple sclerosis (MS) has been extensively reviewed (Croxford et al. 2011; Denic et al. 2011; Pachner 2011; Singhal and Srivastava 2012; Tian et al. 2013). The method is used for evaluation of immunosuppressive properties of drugs (Warford and Robertson 2011; Dasgupta et al. 2011; Paris et al. 2013; Mondal and Pahan 2015).

Procedure

Preparation of the encephalitogen: 3 g of spinal cord from guinea pigs or rats is homogenized with 7.5 ml bidistilled water, 3.8 ml phenol, and 7.5 ml complete Freund's adjuvant under cooling.

Groups of 6–12 male Wistar–Lewis rats with an initial body weight of 130–200 g are used. On day 0, experimental allergic encephalomyelitis is induced by subplantar injection of 0.1 ml of the encephalitogen into the left hind paw. An equal volume of *Bordetella pertussis* vaccine concentrate (200×10^9 organisms/ml) is injected into the same foot. From days 1–2, the animals receive the test compound or vehicle only or the standard drug by oral administration once a day. Body weights of the animals are recorded every second day. The clinical signs of experimental allergic encephalomyelitis consist of ataxia or paresis, i.e., grossly irregular gait and weakness of one or both hind legs followed by flaccid paralysis of the hindquarters, urinary incontinence, fecal impaction, and abdominal wall flaccidity. Animals showing one of these clinical signs are considered positive for the purpose of evaluation.

Evaluation

Starting from day 7, the severity of clinical signs and mortality are determined daily and scored according to the following scheme:

	Score
Per 20 g loss of weight	1
Paralysis of the tail	1
Paralysis of the hind paw	3
Complete paralysis	5
Death	6

Calculation of the Results

The delay of onset of the paralytic symptoms is determined. The total score per day is recorded for treated and control groups. On the day of maximal clinical symptoms occurring among control animals, the total score of the treated groups is compared to the total score of the control group. The percentage change is evaluated.

Doses of 0.5 mg/kg p.o. methotrexate, 1 mg/kg p.o. hydrocortisone, and 2.5 mg/kg p.o. cyclophosphamide were found to be active, whereas

nonsteroidal anti-inflammatory compounds were inactive.

Critical Assessment of the Method

The model of experimental allergic encephalomyelitis in rats is suitable to distinguish between immunosuppressive and anti-inflammatory drugs. Experimental autoimmune encephalomyelitis is considered as a rodent model of the autoimmune disease multiple sclerosis (Pearson et al. 1997; Deng et al. 2002).

Modifications of the Method

The phosphodiesterase inhibitor pentoxifylline was found to prevent induction of experimental autoimmune encephalomyelitis in Lewis rats (Rott et al. 1993).

Martin and Near (1995) studied the protective effect of the interleukin-1 antagonist IL-1ra on experimental allergic encephalomyelitis in Lewis rats.

Experimental autoimmune encephalomyelitis in different strains of mice was described by Heremans et al. (1996), Glabinski et al. (1997), and Liblau et al. (1997).

Baker et al. (1990, 1991, 2000) induced experimental allergic encephalomyelitis in Biozzi AB/H mice by sensitization with 1 mg of mouse spinal cord homogenate emulsified in Freund's complete adjuvant on days 0 and 7. The disease is characterized by relapsing–remitting episodes similar to multiple sclerosis in human beings. Biozzi AB/H mice also develop spasticity and tremor which can be antagonized by cannabinoids.

A chronic relapsing–remitting form of experimental autoimmune encephalomyelitis was induced in the common marmoset *Callithrix jacchus* following a single immunization with human white matter by Massacesi et al. (1995) and Genain and Hauser (1997) and recommended as a new model for multiple sclerosis. This model has been used for histopathological characterization of magnetic resonance imaging-detectable white matter lesions in a primate model of multiple sclerosis by 't Hart et al. (1998, 2004).

Experimental allergic neuritis in several animal species has been described by Waksman and Adams (1955, 1956); King et al. (1983); McCombe et al. (1990), and Nakayasu et al. (1990). This disorder has been considered to show similarities to the Guillain–Barré syndrome in man. The demyelinating process initiated by the injected antigens is a lymphocyte-mediated reaction in which activated macrophages strip myelin off the axons. Hartung et al. (1987) described the adoptive transfer experimental autoimmune neuritis in Lewis rats by injection of P2-reactive T lymphocyte cell lines.

Mix et al. (1992) studied the effect of stilbene-type anion channel blockers on the immune response during experimental allergic neuritis induced by bovine peripheral myelin.

Kojima et al. (1994) investigated the pathogenic potential of autoimmune T cell responses to nonmyelin autoantigens in the Lewis rat using the astrocyte-derived calcium-binding protein S100β as a model nonmyelin autoantigen. In contrast to the experimental autoimmune encephalomyelitis induced by the adoptive transfer of myelin basic protein-specific T line cells, S100β-specific T cell transfer induced intense inflammation not only in the spinal cord but also throughout the entire CNS and also in the uvea and retina of the eye.

Gautam et al. (1992) reported that a polyalanine peptide with only five native basic protein residues induces autoimmune encephalomyelitis in mice. This peptide, called myelin basic protein (MBP) Ac1–11, has been used by several authors for further studies on experimental autoimmune encephalomyelitis (Ratts et al. 1999; Matejuk et al. 2003).

Pearson et al. (1997) reported the induction of a heterogeneous T cell receptor repertoire in (PL/JXSJL/J) F2 mice by myelin basic protein peptide Ac1–11 and its analogue Ac1–11[4A].

Deng et al. (2002) found that expression of the tyrosine phosphatase Src homology 2 domain-containing protein tyrosine phosphatase 1 determines the T cell activation threshold and severity of experimental autoimmune encephalomyelitis.

Maron et al. (2002) investigated the immunological properties of Cop1 (glatiramer acetate) to determine the degree to which its effects were antigen specific using myelin basic protein T cell

receptor transgenic mice. Immunization of these mice fed glatiramer acetate, myelin basic protein, or MBP Ac1–11 resulted in decreased proliferation and IL-2, IL-6, and IFN-γ production and increased secretion of IL-10 and TGF-β in glatiramer acetate-fed animals.

Gilgun-Sherki et al. (2003) reported that riluzole suppresses myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in mice.

Pollak et al. (2003) studied the experimental allergic encephalitis-associated behavioral syndrome and the modulation by anti-inflammatory treatments.

Diab et al. (2004) found that ligands for the PPAR-γ and the retinoid X receptor exert additive anti-inflammatory effects on experimental autoimmune encephalomyelitis. Duckers et al. (1997) studied the effect of a neurotropic treatment on cortical lesion development in experimental allergic encephalomyelitis in rats by longitudinal in vivo magnetic resonance imaging methods.

References and Further Reading

Alvord EC (1984) The challenge: how good a model of MS is EAE today? In: Alvord EC, Kies MW, Suckling AJ (eds) Experimental allergic encephalomyelitis: a useful model for multiple sclerosis. Alan R Liss, New York, pp 3–5

Arnon R (1981) Experimental allergic encephalomyelitis – susceptibility and suppression. Immunol Rev 55:5–30

Baker D, O'Neill JK, Gschmeissner SE, Wilcox CE, Butter C, Turk JL (1990) Induction of chronic relapsing experimental allergic encephalomyelitis in Biozzi mice. J Neuroimmunol 28:261–270

Baker D, O'Neill JK, Turk JL (1991) Cytokines in the nervous system of mice during chronic relapsing experimental allergic encephalomyelitis. Cell Immunol 134:505–510

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman HW, Layward L (2000) Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 202:84–87

Ben-Nun A, Cohen IR (1982) Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. J Immunol 129:303–308

Bolton C, Borel JF, Cuzner ML, Davison AN, Turner AM (1982) Immunosuppression by cyclosporin A of experimental allergic encephalomyelitis. J Neurol Sci 56:147–153

Carlson RP, Baeder WL, Caccese RG, Warner LM, Sehgal SN (1992) Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. Ann N Y Acad Sci 685:86–113

Carlson RP, Hartman DA, Tomchek LA, Walter TL, Lugay JR, Calhoun W, Sehgal SN, Chang JY (1993) Rapamycin, a potential disease-modifying antiarthritic drug. J Pharmacol Exp Ther 266:1125–1138

Chabannes D, Ryffel B, Borel JF (1992) SRI 62–834, a cyclic ether analogue of the phospholipid ET-18-OCH_3, displays long-lasting beneficial effects in chronic relapsing encephalomyelitis in the Lewis rat. Comparison with cyclosporin and (Val^2)-dihydrocyclosporin effects in clinical, functional and histological studies. J Autoimmun 5:199–211

Constantinescu CS, Farooqi N, O'Brien K, Gran B (2011) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis. Br J Pharmacol 164:1079–1106

Croxford AL, Kurschus FC, Waisman A (2011) Mouse model for multiple sclerosis: historical facts and future implications. Biochim Biophys Acta 1812:177–183

Dasgupta S, Mazumder B, Ramani YR, Bhattacharyya SP, Das MK (2011) Evaluation of the role of erythropoietin and methotrexate in multiple sclerosis. Indian J Pharm 43:512–515

Deng C, Minguela A, Hussain RZ, Lovett-Racke AE, Radu C, Ward ES, Racke MK (2002) Expression of the tyrosine phosphatase Src homology 2 domain-containing protein tyrosine phosphatase 1 determines T cell activation threshold and severity of experimental autoimmune encephalomyelitis. J Immunol 168:4511–4518

Denic A, Johnson AJ, Bieber AJ, Warrington AE, Rodriguez M, Pirko I (2011) The relevance of animal models in multiple sclerosis research. Pathophysiology 18:21–29

Diab A, Hussain RZ, Lovett-Racke AE, Chavis JA, Drew PD, Racke MK (2004) Ligands for the peroxisome proliferator-activated receptor-γ and the retinoid X receptor exert additive anti-inflammatory effects on experimental autoimmune encephalomyelitis. J Neuroimmunol 148:116–126

Duckers HJ, Muller HJ, Verhaagen J, Nicolay K, Gispen WH (1997) Longitudinal in vivo magnetic resonance imaging studies in experimental allergic encephalomyelitis : effect of a neurotropic treatment on cortical lesion development. Neuroscience 77: 1163–1173

Feurer C, Chow LH, Borel JF (1988) Preventive and therapeutic effects of cyclosporin and valine^2-dihydro-cyclosporin in chronic relapsing experimental allergic encephalomyelitis in the Lewis rat. Immunology 63:219–223

Gautam AM, Pearson CI, Smilek DE, Steinman L, McDevitt HO (1992) A polyalanine peptide with only five native basic protein residues induces autoimmune encephalomyelitis. J Exp Med 176:605–609

Genain CP, Hauser SL (1997) Creation of a model for multiple sclerosis in *Callithrix jacchus* marmosets. J Mol Med 75:187–197

Gilgun-Sherki Y, Panet H, Melamed E, Offen D (2003) Riluzole suppresses experimental autoimmune encephalomyelitis: implications for the treatment of multiple sclerosis. Brain Res 989:196–204

Glabinski AR, Tani M, Strieter RM, Tuohy VK, Ransohoff RM (1997) Synchronous synthesis of α- and β-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. Am J Pathol 150:617–630

Hartung HP, Schäfer B, Fierz W, Heininger K, Toyka KV (1987) Cyclosporin A prevents P2 T cell line-mediated experimental autoimmune neuritis (AT-EAN) in rat. Neurosci Lett 83:195–200

Heremans H, Dillen C, Groenen M, Martens E, Billiau A (1996) Chronic relapsing experimental autoimmune encephalomyelitis in mice: enhancement by monoclonal antibodies against interferon-gamma. Eur J Immunol 26:2393–2398

Hinrichs DJ, Wegmann KW, Peters BA (1983) The influence of cyclosporin A on the development of actively induced and passively transferred experimental allergic encephalomyelitis. Cell Immunol 77:202–209

King RHM, Craggs RI, Gross MLP, Tompkins C, Thomas PK (1983) Suppression of experimental allergic neuritis by cyclosporin A. Acta Neuropathol (Berl) 59:262–268

Kojima K, Berger T, Lassmann H, Hinze-Selch D, Zhang Y, Gehrmann J, Reske K, Wekerle H, Linington C (1994) Experimental autoimmune panencephalitis and uveoretinitis transferred to the Lewis rat by T lymphocytes specific for the S100 β molecule, a calcium binding protein of astroglia. J Exp Med 180:817–829

Levine S, Sowinski R (1977) Suppression of the hyperacute form of experimental allergic encephalomyelitis by drugs. Arch Int Pharmacodyn Ther 230:309–318

Liblau R, Steinman L, Brocke S (1997) Experimental autoimmune encephalomyelitis in IL-4 deficient mice. Int Immunol 9:799–803

Maron R, Aj S, Hoffmann E, Komagata Y, Weiner HL (2002) Oral tolerance to copolymer 1 in myelin basic protein (MBP) TCR transgenic mice: cross-reactivity with MBP-specific TCR and differential induction of anti-inflammatory cytokines. Int Immunol 14:131–138

Martin D, Near SL (1995) Protective effect of the interleukin-1 antagonist (IL-1ra) on experimental allergic encephalomyelitis in rats. J Neuroimmunol 61:241–245

Massacesi L, Genain CP, Lee-Parritz D, Letvin NL, Canfield D, Hauser SL (1995) Active and passively induced experimental autoimmune encephalomyelitis in common marmosets: a new model of multiple sclerosis. Ann Neurol 37:519–530

Matejuk A, Hopke C, Dwyer J, Subramanian S, Jones RE, Bourdette DN, Vandenbark AA,

Offner H (2003) CNS gene expression pattern associated with spontaneous experimental autoimmune encephalomyelitis. J Neurosci Res 73:667–678

McCombe PA, van der Kreek SA, Pender MP (1990) The effects of prophylactic cyclosporin A on experimental allergic neuritis (EAN) in the Lewis rat. Induction of relapsing EAN using low dose of cyclosporin A. J Neuroimmunol 28:131–140

McFarlin DF, Blank SE, Kibler RF, McKneally S, Shapira R (1973) Experimental allergic encephalomyelitis in the rat: response to encephalitogenic proteins and peptides. Science 179:478–483

Mix E, Correale J, Olsson T, Solders G, Link H (1992) Effect of stilbene-type anion channel blockers on the immune response during experimental allergic neuritis. Immunopharmacol Immuntoxicol 14:579–609

Mondal S, Pahan K (2015) Cinnamon ameliorate experimental allergic encephalomyelitis in mice via regulatory T cells: implications for multiple sclerosis therapy. PLoS One 10: e0116566

Nakayasu H, Ota K, Tanaka H, Irie H, Takahashi H (1990) Suppression of actively induced and passively transferred experimental allergic neuritis by cyclosporin A. J Neuroimmunol 26:219–227

Pachner AR (2011) Experimental models of multiple sclerosis. Curr Opin Neurol 24:291–299

Paris D, Beaulieu-Abdelahad D, Mullan M, Ait-Ghezala G, Mathura V, Bachmeier C, Crawford F, Mullan MJ (2013) Amelioration of experimental autoimmune encephalomyelitis by anatabine. PLoS One 8:e55392

Pearson CI, Smilek DE, Danska JS, McDevitt HO (1997) Induction of a heterogeneous TCR repertoire in (PL/JXSJL/J) F2 mice by myelin basic protein peptide Ac1–11 and its analog Ac1–11[4A]. Mol Immunol 14:781–792

Pollak Y, Ovadia H, Orion E, Yimiya R (2003) The EAE-associated behavioral syndrome: II. Modulation by anti-inflammatory treatments. J Neuroimmunol 137:100–108

Polman CH, Matthaei I, de Groot CJA, Koetsier JC, Sminia T, Dijkstra CD (1988) Low-dose cyclosporin A induces relapsing remitting experimental allergic encephalomyelitis in the Lewis rat. J Neuroimmunol 17:209–216

Ratts RB, Arredono LR, Bittner P, Perrin PJ, Lovett-Racke AE, Racke MK (1999) The role of CTLA-4 in tolerance induction and T cell differentiation in experimental autoimmune encephalomyelitis.: i.p. antigen administration. Int Immunol 11:1881–1888

Rivers TM, Sprunt DH, Berry GP (1933) Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. J Exp Med 58:39–53

Rosenthale ME, Datko LJ, Kassarich J, Schneider F (1969) Chemotherapy of experimental allergic encephalomyelitis (EAE). Arch Int Pharmacodyn Ther 179:251–275

Rott O, Cash E, Fleischer B (1993) Phosphodiesterase inhibitor pentoxifylline, a selective suppressor of T helper type 1-but not type 2-associated lymphokine production, prevents induction of experimental autoimmune encephalomyelitis in Lewis rats. Eur J Immunol 23:1745–1751

Schuller-Levis GB, Kozlowski PB, Wisniewski HM (1986) Cyclosporin A treatment of an induced attack in a chronic relapsing model of experimental allergic encephalomyelitis. Clin Immunol Immunopathol 40:244–252

Singhal M, Srivastava P (2012) Experimental autoimmune encephalomyelitis model for discovery of new therapy for multiple sclerosis. Glob J Pharmacol 6:208–215

't Hart BA, Bauer J, Muller HJ, Melchers B, Nicolay K, Brok H, Bontrop RE, Lassmann H, Massacesi L (1998) Histopathological characterization of magnetic resonance imaging-detectable white matter lesions in a primate model of multiple sclerosis. A correlative study in the experimental autoimmune encephalomyelítis model in common marmosets (*Callithrix jacchus*). Am J Pathol 153:649–663

't Hart BA, Vogel J, Bauer J, Brok HPM, Blezer E (2004) Non-invasive measurement of brain damage in a primate model of multiple sclerosis. Trends Mol Med 10:85–91

Tian DH, Perera CJ, Moalem-Taylor G (2013) Neuropathic pain in animal models of nervous system autoimmune diseases. Mediators Inflamm 2013:298326. doi:10.1155/2013/298326

Waksman BH, Adams RD (1955) Allergic neuritis: an experimental disease of rabbits induced by the injection of peripheral nervous tissue and adjuvants. J Exp Med 102:213–234

Waksman BH, Adams RD (1956) A comparative study of experimental allergic neuritis in rabbit, guinea-pig and mouse. J Neuropathol Exp Neurol 15:293–374

Warford J, Robertson GS (2011) New methods for multiple sclerosis drug discovery. Expert Opin Drug Discov 7:689–699

Acute Graft-Versus-Host Disease (GVHD) in Rats

Purpose and Rationale

The intravenous injection of a mixture of parental splenocytes into healthy inbred F_1-rats results in graft-versus-host (GVH)-induced immune abnormalities. This is due to T lymphocytes in the donor inoculum that recognize the major histocompatibility alloantigens expressed by the F_1-animals. The host F_1 T cells are genetically unable to recognize antigens of the parental donor as foreign; thus, the response involves only donor recognition of host and not host recognition of donor. The ensuing immune abnormalities lead to clinical symptoms of an acute, lethal GVH-disease (GVHD), i.e., profound immunodeficiency, anemia, hypogammaglobulinemia, and runting.

Procedure

Three- to 4-month-old male F_1-hybrid rats of the inbred strains Lewis (Rt-1 l) and Brown Norway (BN, Rt-1n) (Zentralinstitut für Versuchstierkunde, Hannover, Germany) are used as hosts for cell grafts from the Lewis parental strain. The bone marrow cells are obtained by flushing hind femur bone shafts with culture medium. These cells are then pooled together with spleen cells (ratio 2 bones/1 spleen). The cell viability, determined by trypan exclusion, has to be more than 90 %. Each recipient is injected with about 40×10^7 cells in a 1.5 ml suspension volume. The route of injection is the penis vein, allowing an optimal control of correct intravenous application.

Prophylactic Drug Application

For this experiment, two groups of 6 F_1-hybrids each are injected with the abovementioned bone marrow/spleen cell suspension. One group receives the test drug orally and daily until the end of the experiment, homogeneously suspended in 1 % carboxymethylcellulose (CMC) solution. The other group receives CMC alone and, thus, serving as the GVHD control group. The experiment is terminated 2 weeks after disease induction, i.e., 1 week after the first appearance of GVHD symptoms. All animals are sacrificed and clinical aspects documented; spleens weighed; histology of the skin, liver, spleen, and lymph nodes performed; and organs photographed.

Therapeutic Drug Application

In this experiment, rats are separated into four groups and treatment begins with the first sign of GVHD symptoms (beginning of the second week). Because of the expected, greater therapeutic difficulty, the daily dose of the test drug has to be doubled, again for 2 weeks duration.

The experiment is terminated either by sacrificing those rats that are too sick to be able to move around the cage or at the end of the 4-week observation period, regardless of the clinical condition of the animals. The clinical-chemical parameters are determined by routine procedures conducted with a Hitachi autotechnicon.

Evaluation

The tested parameters of therapeutic success or disease, respectively, are survival rate (%), spleen weight (g), and body weight (g) as well as clinical-chemical parameters (bilirubin, alkaline phosphatase, creatinine, white cell count) after 2 and 3 weeks.

Modifications of the Method

Gelpi et al. (1994) established a chronic graft-versus-host disease in (C5BL/10 × DBA/2) F_1 mice with an injection of lymphoid cells from

the parent DBA/2 strain. Most of the animals developed antibodies against transfer RNA/protein particles.

Mosier et al. (1988) reported transplantation of human peripheral blood lymphocytes (PBL) into severe combined immunodeficient (SCID) mice to construct hu-PBL-SCID mice. Kim et al. (1997) suggested these mice for routine immunotoxicity investigations using lymph nodes of intestines as the lymphocyte sources.

Ford et al. (1970) and Schorlemmer et al. (1997, 1998) used the popliteal lymph node assay to study the local graft-versus-host reaction. The test is based on the enlargement of the draining popliteal lymph nodes as a result of injecting immunocompetent cells (1×10^8 parental Lewis spleen cells) into the hind foot pad of Lewis × Brown Norway F1 recipients. The reaction is measured at day 6 after challenge as a gain in lymph node weights.

Xu et al. (2010) explored the effects of both rapamycin and tacrolimus in the model measuring animal survival after liver transplantation and reporting a differential effect on survival between the two drugs. Xia et al. (2013) investigated the effects of Trichostatin A (TSA) in the rat model of liver transplantation and concluded that TSA did not abrogate acute graft-versus-host disease due to a downregulation of regulatory T cells.

References and Further Reading

Bartlett RR, Dimitrijevic M, Mattar T, Zielinski T, Germann T, Rüde E, Thoenes GH, Küchle CCA, Schorlemmer HU, Bremer E, Finnegan A, Schleyerbach R (1991) Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of autoimmune disorders and reactions leading to transplantation rejection. Agents Actions 32:11–21

Bartlett RR, Anagnostopulos H, Zielinski T, Mattar T, Schleyerbach R (1993) Effects of leflunomide on immune responses and models of inflammation. Springer Semin Immunopathol 14:381–394

Caballero-Velázquez T, Sánchez-Abarca LI, Gutierrez-Cosio S, Blanco B, Calderon C, Herrero C, Carrancio S, Serrano C, del Cañizo C, San Miguel JF, Pérez-Simón JA (2012) The novel combination of sirolimus and bortezomib prevents graft-versus-host disease but maintains the graft-versus-leukemia effect after allogeneic transplantation. Haematologica 97:1329–1337

Deol A, Ratanatharathorn V, Uberti JP (2011) Pathophysiology, prevention, and treatment of acute graft-versus-host disease. Transpl Res Risk Manag 3:31–44

Ford WL, Burr W, Simonsen G (1970) A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. Transplantation 10:258

Gelpi C, Martinez MA, Vidal S, Targoff IN, Rodriguez-Sanchez JL (1994) Antibodies to a transfer RNA-associated protein in a murine model of chronic graft versus host disease. J Immunol 152:1989–1999

Jones KR, Kang EM (2015) Graft versus host disease: new insights into A_{2A} receptor agonist therapy. Comp Struct Biotechnol J 13:101–105

Kim HM, Han SB, Hong DH, Yoo BS, Oh GT (1977) Limitation of Hu-PBL-scid mouse model in direct application to immunotoxicity assessment. J Pharmacol Toxicol Methods 37:83–89

Küchle CCA, Thoenes GH, Langer KH, Schorlemmer HU, Bartlett RR, Schleyerbach R (1991) Prevention of kidney and skin graft rejection in rats by leflunomide, a new immunomodulating agent. Transplant Proc 23:1083–1086

Leventhal J, Huang Y, Xu H, Goode I, Ildstad ST (2012) Novel regulatory therapies for prevention of Graft-versus-host disease. BMC Med 10:48

Mosier DE, Gulizia RJ, Baird SM, Wilson DB (1988) Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature 335:256–259

Mrowka C, Thoenes GH, Langer KH, Bartlett RR (1994) Prevention of acute graft versus host disease (GVHD) in rats by the immunomodulating drug leflunomide. Ann Hematol 68:195–199

Murase N, Demetris AJ, Woo J, Tanabe M, Furuya T, Todo S, Starzl TE (1993)

Graft-versus-host disease after Brown Norway-to-Lewis and Lewis-to-Brown Norway rat intestinal transplantation under FK 506. Transplantation 55:1–7

Punj S, Koppaparu P, Jang HS, Phillips JL, Pennington J, Rohlman D, O'Donnell E, Iverson PL, Kolluri SK, Kerkvliet NI (2014) Benzimidazoisoquinolines: a new class of rapidly metabolized aryl hydrocarbon receptor (AhR) ligands that induce AhR-dependent tregs and prevent murine graft-versus-host disease. PLoS One. doi: 10.1371/journal.pone.0088726

Renkonen R, Häyry P (1984) Bone marrow transplantation in the rat. I. Histologic correlation and quantification of cellular infiltrates in acute graft-versus-host disease. Am J Pathol 117:462–470

Schorlemmer HU, Seiler FR, Bartlett RR (1993) Prolongation of allogenetic transplanted skin grafts and induction of tolerance by leflunomide, a new immunosuppressive isoxazol derivative. Transplant Proc 25:763–767

Schorlemmer HU, Kurrle R, Bartlett R (1997) The new immunosuppressants, the malononitrilamides MNA 279 and MNA 715, inhibit various graft-vs.-host diseases (GvHD) in rodents. Drugs Exp Clin Res 23:167–173

Schorlemmer HU, Kurrle R, Schleyerbach R (1998) A77–1726, leflunomide's active metabolite, inhibits in vivo lymphoproliferation in the popliteal lymph node assay. Int J Immunother 14:205–211

Shaffer D, Muanza T, Blakely ML, Simpson MA, Monaco AP (1993) Prevention of graft-versus-host-disease by RS-61443 in two different rodent models. Transplantation 55:221–223

Strober S (2014) Path to clinical transplantation tolerance and prevention of graft versus host disease. Immunol Res 58:240–248

Thoenes GH, Sitter T, Langer KH, Bartlett RR, Schleyerbach R (1989) Leflunomide (HWA 486) inhibits experimental autoimmune tubulointerstitial nephritis in rats. Int J Immunopharmacol 11:921–929

Wakely E, Oberholser JH, Corry RJ (1990) Elimination of acute GVHD and prolongation of rat pancreas allograft survival with DST, cyclosporine, and spleen transplantation. Transplantation 49:241–245

Xia X, Liang C, Liu H, Xue F, Hu Q, Chen W, Ma T, Zhang Y, Bai X, Liang T (2013) Effects of trichostatin A in a rat model of acute graft-versus-host disease after liver transplantation. Transplantation 96:25–33

Xu G, Wang L, Chen W, Xue F, Bai X, Liang L, Shen X, Zhang M, Xia D, Liang T (2010) Rapamycin and Tacrolimus differentially modulate acute graft-versus-host disease in rats after liver transplantation. Liver Transpl 16:357–363

Zeiser R (2014) Animal models of graft-versus-host disease. Hematol Educ 8:359–366

Influence on SLE-Like Disorder in MRL/lpr Mice

Purpose and Rationale

Systemic lupus erythematosus (SLE) is an autoimmune disease in man that affects multiple body organs and is characterized by the development of certain types of self-antigens. Primarily, the antibodies formed against double-stranded DNA (dsDNA), the most prevalent in this ailment, complex together and, with complement, deposit in the small blood vessels, leading to widespread vasculitis. MRL Mpf lpr/lpr (MRL/lpr) mice spontaneously develop a severe disease with many symptoms very similar to human SLE, i.e., hypergammaglobulinemia and glomerulonephritis (Theofilopoulos and Dixon 1981). Recent years have seen the development of numerous animal models of skin disease which have assisted the discovery of potential new drugs for clinical testing (Rottman and Willis 2010; Avci et al. 2013) which in part have allowed progression of a number of small-molecule candidate drugs (Kyttaris et al. 2013; Markopoulou and Kyttaris 2013).

Procedure

Female MRL/lpr mice (originally from Jackson Laboratories, USA), displaying distinct symptoms of SLE (between 12 and 13 weeks of age), are randomized and divided into groups of

12 animals each. At this age, the animals have already clinical manifestations of the SLE-like illness, as determined by the disease index, but have not yet developed proteinuria. Animals with early symptoms of disease are treated with various drugs, e.g., leflunomide, cyclosporine A, azathioprine, cyclophosphamide, or prednisolone, for 11 weeks, and the survival rate and disease index of these animals are followed for 24 weeks. The disease index and urine protein level are determined once weekly.

Disease Index

The subsequent clinical parameters are taken into consideration:

1. Ears: reddening of the skin, deterioration of the pinna
2. Nose: loss of hair, wasting of the skin
3. Lymph nodes: detection of swollen lymph nodes on any part of the body, especially the neck and extremities
4. Fur: general condition of fur (e.g., shabby, mangy, etc.), loss of hair
5. Skin: inflammation of the skin, scab, and/or granuloma formation
6. Eyes: exophthalmos, deterioration due to inflammation, tumor formation around the eye, swelling of the eyelid with eventual closure of the eye
7. Paws: reddening of the skin, swelling of the paw

Evaluation

A score for each of the above-described parameters is given according to the severity of the symptoms as follows:

Points for Clinical Index

Involvement	Detectable	Moderate	Severe
Ears (each)	0.5	1.0	1.5
Nose	1.0	2.0	3.0
Lymph node (each)	1.0	2.0	3.0
Fur	1.0	2.0	3.0
Skin	1.0	2.0	3.0
Eyes (each)	1.0	2.0	3.0
Paws (each)	0.5	1.0	1.5

Body weight (one point for 5 g difference from week to week)

The determination of the disease index is performed, weekly, by the same individual, but without knowledge of the group being evaluated. The points, for each animal, are registered and the total score, of each group, summarized. The average score for the group is calculated, and significance between the experimental group and the untreated diseased group is determined using the Student's *t*-test.

Proteinuria

Pooled urine is collected from each experimental group and the amount of protein in the urine is calculated.

Modifications of the Method

In addition to a lupus-like syndrome and massive T cell proliferation, MRL-1pr/1pr (MRL/1) mice develop an arthritic process very similar serologically and histologically to human rheumatoid arthritis. Boissier et al. (1989) found that in these animals, mouse type II collagen is antigenic, but not arthritogenic.

Holmdahl et al. (1991) studied the involvement of macrophages and dendritic cells in synovial inflammation of collagen-induced arthritis in DBA/1 mice and spontaneous arthritis in MRL/lpr mice.

Rordorf-Adam et al. (1985) used serum amyloid P component and autoimmune parameters in the assessment of arthritis in MRL/lpr/lpr mice.

Furukawa et al. (1996) studied the autoimmune disease-prone genetic background in relation to Fas defect in MRL/lpr mice.

Kanno et al. (1992) found spontaneous development of pancreatitis in the MRL/Mp strain of mice.

Kusakari et al. (1992) compared hearing acuity and inner ear disorders of MRL/lpr mice with those of BALB/c mice and found a significantly higher auditory brain stem response threshold. They recommended this as a model of sensorineural hearing loss.

Bundick and Eady (1992) investigated the effects of an immunosuppressive agent on the development of spontaneous lupus disease in female NZBW F1-hybrid mice.

Walker et al. (1996) reported a powerful suppressive effect of testosterone on the autoimmune disease analogous to systemic lupus erythematodes spontaneously developed by F1-hybrids of New Zealand Black (NZB) × New Zealand White (NZW) mice. A model was developed in which NZB dams carrying NZB/NZW fetuses were treated with testosterone in a dose adequate to masculinize the external genitalia in female fetuses.

Zoja et al. (1998) investigated bindarit, a compound devoid of immunosuppressive properties, in NZB/W F1 hybrid mice developing an immune complex glomerulonephritis with proteinuria and progression to renal insufficiency.

Kiberd and Stadnyk (1995) studied the role of endogenous interleukin-1 in established lupus nephritis in MRL-lpr/lpr mice by administration of the IL-1 receptor antagonist IL-1ra.

Gleichmann et al. (1982) and Schorlemmer et al. (1997) induced a systemic lupus erythematodes-like disease in mice by abnormal T and B cell cooperation. A chronic graft-versus-host reaction with the pathologic symptoms of severe glomerulonephritis is induced in B6D2 (C5Bl/6 × DBA/2) F1 hybrid mice receiving four i.v. injections (one per week) of 1×10^8 parental lymphoid spleen cells from DBA/2 donors. The inoculation of splenocytes into the BDF1 hybrid mice results in the development of a chronic GvH reaction with lymphoid hyperplasia, autoantibody production, and immune complex glomerulonephritis.

Chan et al. (1995) described ocular changes occurring in mice with experimental lupus erythematodes. The ocular disease is characterized by bilateral subacute and chronic inflammation of the eyelids (blepharitis) and hypertrophic meibomian glands. The severity of the ocular changes is strain dependent. The authors recommend this experimental eye disease as an animal model for chronic blepharitis in humans.

The changes of lacrimal and salivary glands found in MRL/lpr mice and other mouse strains with autoimmune disorders were also regarded as model of Sjögren's syndrome in human (Sullivan and Edwards 1997; Toda et al. 1999).

The MRL-lpr mouse model has been used to provide cognitive dysfunction in neuropsychiatric systemic lupus erythematosus (Jeltsch-David and Muller 2014), and peptide microarray technology has been developed which may facilitate diagnosis and early detection of CNS-SLE (Williams et al. 2014).

Several studies have investigated the effects of T cell modulation in the MRL/lpr model (Richard et al. 2013; Shinsuke and Hiroshi 2013), and the role of peptidylarginine deiminase and NET formation has been investigated in the MRL/lpr model (Knight et al. 2014).

An assessment of the value of murine lupus models for translation of findings into the clinic (Bender et al. 2014) has highlighted the individuals' strengths of the various models available.

References and Further Reading

Avci P, Sadasivam M, Gupta A, De Melo WCMA, Huang Y-Y, Yin R, Rakkiyappan C, Kumar R, Otufowora A, Nyame T, Hamblin MR (2013) Animal models of skin disease for drug discovery. Expert Opin Drug Discov 8:331–355

Bartlett RR, Popovic S, Raiss RX (1988) Development of autoimmunity in MRL/lpr mice and the effect of drugs on this murine disease. Scand J Rheumatol Suppl 75:290–299

Bartlett RR, Mattar T, Weithmann U, Anagnostopulos H, Popovic S, Schleyerbach R (1989) Leflunomide (HWA 486): a novel immunorestoring drug. In: Lewis AJ, Doherty NS, Ackerman NR (eds) Therapeutic approaches to inflammatory diseases. Elsevier Science, New York, pp 215–228

Bender AT, Wu Y, Cao Q, Ding Y, Oestreicher J, Genest M, Akare S, Ishizaka ST, Mackey MF (2014) Assessment of the translational value of mouse lupus models using clinically relevant biomarkers. Transl Res 163:515–532

Boissier MC, Texier B, Carlioz A, Fournier C (1989) Polyarthritis in MRL-1pr/1pr mice: mouse type II collagen is antigenic, but not arthritogenic. Autoimmunity 4:31–41

Bundick RV, Eady RP (1992) The effects of CP 17193, an immunosuppressive pyrazoloquinoline, on the development of spontaneous lupus disease in NZBW F_1 hybrid mice. Clin Exp Immunol 89:179–184

Carlson RP, Baeder WL, Caccese RG, Warner LM, Sehgal SN (1992) Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. Ann N Y Acad Sci 685:86–113

Chan CC, Gery I, Kohn LD, Nussenblatt RB, Mozes E, Singer SD (1995) Periocular inflammation in mice with experimental systemic lupus erythematodes. A new experimental blepharitis and its modulation. J Immunol 154:4830–4835

Furukawa F, Kanauchi H, Wakita H, Tokura Y, Tachibana T, Horiguchi Y, Imamura S, Ozaki S, Takigawa M (1996) Spontaneous autoimmune skin lesions of MRL/n mice: autoimmune disease-prone genetic background in relation to Fas-defect MRL/lpr mice. J Invest Dermatol 107:95–100

Gleichmann E, van Elven EH, van der Veen JPW (1982) A systemic lupus erythematodes (SLE)-like disease in mice induced by abnormal T- and B-cell cooperation. Preferential formation of antibodies characteristic of SEL. Eur J Immunol 12:152

Gunn HC, Hiestand PC (1988) Cyclosporine A and cyclosporine G enhance IgG rheumatoid factor production in MRL/lpr mice. Transplant Proc 20(Suppl 4):238–242

Holmdahl R, Tarkowski A, Jonsson R (1991) Involvement of macrophages and dendritic cells in synovial inflammation of collagen induced arthritis in DBA/1 mice and spontaneous arthritis in MRL/Lpr mice. Autoimmunity 8:271–280

Jeltsch-David H, Muller S (2014) Neuropsychiatric systemic lupus erythematosus and cognitive dysfunction: the MRL/lpr mouse strain as a model. Autoimmun Rev 13:963–973

Kanno H, Nose M, Itoh J, Taniguchi Y, Kyogoku M (1992) Spontaneous development of pancreatitis in the MRL/Mp strain of mice in autoimmune mechanism. Clin Exp Immunol 89:68–73

Kiberd BA, Stadnyk AW (1995) Established murine lupus nephritis does not respond to exogenous interleukin-1 receptor antagonist: a role for the endogenous molecule? Immunopharmacology 30:131–137

Konya C, Kyttaris VC (2013) T cells as treatment targets in systemic lupus erythematosus. Rheumatol Curr Res 3:120. doi: 10.4172/2161-1149.1000120

Knight JS, Subramanian V, O'Dell AA, Yalavarhi S, Zhao W, Smith CK, Hodgin JB, Thompson PR, Kaplan MJ (2014) Peptidylarginine deaminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. Ann Rheum Dis. doi: 10.1136/annrheumdis-2014-205365

Kusakari C, Hozawa K, Koike S, Kyogoku M, Takasaka T (1992) MRL/MP-lrp/Lrp mouse as a model of immune-induced sensorineural hearing loss. Ann Otol Rhinol Laryngol 101:82–86

Kyttaris VC, Kampagianni O, Tsokos GC (2013) Treatment with Anti-Interleukin 23 Antibody Ameliorates Disease in Lupus-Prone Mice. BioMed Res Int 2013. Article ID 861028, 5 pp. doi:10.1155/2013/861028

Marcinko K, Parsons T, Lerch JP, Sled JG, Sakic B (2012) Effects of prolonged treatment with memantine in the MRL model of central nervous system lupus. Clin Exp Neuroimmunol 3:116–128

Markopoulou A, Kyttaris VC (2013) Small molecules in the treatment of systemic lupus erythematosus. Clin Immunol 148:359–368

Richard EM, Thiyagarajan T, Bunni MA, Basher F, Roddy PO, Siskind LJ, Nietart PJ, Nowling TK (2013) Reducing FLI1 levels in the MRL/lpr lupus mouse model impacts T cell function by modulating glycosphingolipid metabolism. PLoS One. doi: 10.1371/kournal.pone.0075175

Rordorf-Adam C, Serban D, Pataki A, Gruninger M (1985) Serum amyloid P component and autoimmune parameters in the assessment of arthritis in MRL/lpr/lpr mice. Clin Exp Immunol 61:509–516

Rottman JB, Willis CR (2010) Mouse models of systemic lupus erythematosus reveal a complex pathogenesis. Vet Pathol 47:664–676

Schorlemmer HU, Dickneite G (1992) Preclinical studies with 15-deoxyspergualin in various animal models for autoimmune diseases. Ann N Y Acad Sci 685:155–174

Schorlemmer HU, Dickneite G, Enßle KH (1995) Immunoregulation of murine SLE-like diseases by interleukin-4-receptor. Lupus 4 (Suppl 2):8

Schorlemmer HU, Kurrle R, Bartlett R (1997) The new immunosuppressants, the malononitrilamides MNA 279 and MNA 715, inhibit various graft-vs.-host diseases (GvHD) in rodents. Drugs Exp Clin Res 23:167–173

Shinsuke N, Hiroshi I (2013) Over-expression of Epstein-Barr virus-induced gene 3 protein (EIB3) in MRL/lpr mice suppresses their lupus nephritis by activating regulatory T cells. Autoimmunity 46:446–454

Sullivan DA, Edwards JA (1997) Androgen stimulation of lacrimal gland function in mouse models of Sjögren's syndrome. J Steroid Biochem Mol Biol 60:237–245

Theofilopoulos AN, Dixon FJ (1981) Etiopathogenesis of murine SLE. Immunol Rev 55:179–216

Toda I, Sullivan BD, Rocha EM, Da Silveira LA, Wickham LA, Sullivan DA (1999) Impact of gender on exocrine gland inflammation in mouse models of Sjögren's syndrome. Exp Eye Res 69:355–366

Walker SE, Keisler LW, Caldwell CW, Kier AB, Vom Saal FS (1996) Effects of altered prenatal hormonal environment on expression of autoimmune disease in NZB/NZW mice. Environ Health Perspect 104(Suppl 4):815–821

Williams S, Stafford P, Hoffman SA (2014) Diagnosis and early detection of CNS-SLE in MRL/lpr mice using peptide microarrays. BMC Immunol 15:23

Zoja C, Corna D, Benedetti G, Morigi M, Donadelli R, Guglielmotti A, Pinza M, Bertani T, Remuzzi G (1998) Bindarit retards renal disease and prolongs survival in murine lupus autoimmune disease. Kidney Int 53:726–734

Prevention of Experimentally Induced Myasthenia Gravis in Rats

Purpose and Rationale

Myasthenia gravis is an organ-specific autoimmune disease in man that results in skeletal muscles' weakness. Typically, the sufferer has drooping eyelids, a blank facial expression, and weak, hesitant speech. This is due to the formation of autoantibodies against the nicotinic acetylcholine receptor (AChR). The formation of autoantibodies to acetylcholine's receptor leads to a gradual destruction of the receptors in skeletal muscles that receive nerve impulses and initiate muscle contractions. As a result, affected muscles fail to respond or react only weakly to nerve signals.

Experimental myasthenia gravis (EMG) can be induced in rats by injecting them with heterologous AChR or with recombinant α-subunits (two) of the AChR (portion of the AChR to which acetylcholine mainly binds) (Lennon et al. 1991), and the utility of clinical trials to guide the use of animal models has been recently addressed (Punga et al. 2015). The animals display symptoms of myasthenia (electrophysiological evidence of altered neuromuscular function) and detectable antireceptor antibodies. The severity of the disease can vary, but most animals display, at the very least, a weakness and fatigability of foot grip. The disease gradually leads to abnormal gait and eventually the inability of the animals to walk or even right themselves.

Procedure

Female rats of AO strain, 6–10 weeks old, are used. Three groups of rats are included in the experiment:

1. Immunized with acetylcholine receptor (AChR) protein and treated with test drug.
2. Immunized with AChR protein without drug.
3. Nonimmunized, non-treated control rats. The test drug is applied per os daily. First dose is administered on the day of immunization and the last on the day of sacrifice.

Immunization with AChR Protein

AChR protein isolated from *Torpedo marmorata* is emulsified with complete Freund's adjuvant, and 100 μg/rat is injected intradermally in the hind foot pad. As additional adjuvant, 2.6×10^{10} *Bordetella pertussis* microorganism is administered simultaneously by intramuscular injection in the hind leg.

Antibody Determination

Anti-AChR-protein antibodies are measured by enzyme-linked immunosorbent assay (ELISA) as described by Norcross et al. (1980). AChR protein is diluted to a final concentration of 2.5 μg/ml in 0.05 M carbonate buffer, pH 9.6. Two hundred ml of this solution is placed in each well of a microtitration plate (Flow Laboratories Inc.). After an overnight incubation at 4 °C, the plates are washed thoroughly with 0.01 M phosphate-buffered saline (PBS) solution containing 0.05 % Tween 20 (Sigma) subsequently referred to as PBS/T. Sera from all groups of rats are serially diluted in PBS/T, and 200 μl is added to each micron well except in the background row (control row) and incubated at 4 °C for 2 h. After washing, 200 μl of 1:1,000 diluted peroxidase-conjugated goat anti-rat immunoglobulin (Sera Lab. Sussex, England) in PBS/T is added to the micron wells and incubated for an additional 60 min at 4 °C. After plates are washed, 200 μl of substrate-citrate buffer and 0.2 μl of 10 % H_2O_2 are added and then incubated in the dark at room temperature for 30 min. The reaction is stopped by addition of 50 μl of 2 M H_2SO_4 and the OD determined by using Titert Multiscan.

Two-Color Flow Cytometry

Thymic cell suspensions are obtained by mincing tissue and passing it through 80-mm stainless mesh. After being washed three times in PBS, the cells are resuspended in PBS at a cell density of 10^7 viable cells/ml. The cell viability is determined by the trypan blue exclusion test. Erythrocytes are removed by addition of ammonium chloride. Cell staining and flow cytometric analyses are done as described by Itoyama et al. (1989). Thymocyte subsets expressing CD4 and/or CD8 molecules are defined by staining with monoclonal antibodies obtained from Serotec, Oxford, England: phycoerythrin (PE)-conjugated anti-W3/25 (CD4) and fluorescein isothiocyanate (FITC)-conjugated anti-MRC OX8 (CD8). Two $\times 10^5$–1×10^6 cells suspended in 100 ml of PBS are exposed sequentially for 30 min to FITC-conjugated anti-CD8 and PE-conjugated anti-CD4 monoclonal antibodies. Isotype-matched control monoclonal antibodies are used to prove the specificity of binding. Cell analysis is performed using FACScan flow cytometer from Becton Dickinson. One $\times 10^4$ events per sample are analyzed by Consort 30 and Lysis software. All data are collected and displayed on a log scale of increasing green and orange fluorescence intensity. This is presented as two-dimensional contour maps and as percentage of thymocytes by integrating counts in selected areas of the contour plots.

Stereologic Analysis of Thymuses

Thymuses of animals of all groups are prepared for light microscopic analysis. For this purpose, thymus tissue is fixed in Carnoy's solution, embedded in paraffin, and 3–5-μm-thin sections are stained with hematoxylin and eosin. Cortex and medulla are analyzed stereologically using the point counting method described by Weible (1963). Volume density (*Vv*) of the examined structures is determined by the following equation: $Vv = Pi/Pt$, where *Pi* represents the number of points of the examined structure and *Pt* the total number of points. *Vv* refers to the volume fraction, i.e., volume of a feature per unit test volume (Tascaland Vaughn-Williams 1981).

Evaluation

EMG is evaluated clinically by daily examination of muscle weakness and scored as follows:

+ = weakness of grip with fatigability
++ = abnormality of gait
+++ = inability to walking and righting

Immediately after appearance of clinical signs of EMG, rats are sacrificed, and blood and thymuses are taken for determination of anti-AChR-

protein antibodies and histological analysis of thymuses and thymocyte subsets, respectively.

Statistical analysis of data is performed by Student's *t*-test (data of stereological analysis) and Mann–Whitney *U*-test (results of flow cytometric analysis of thymocyte subsets).

Modifications of the Method

McIntosh and Drachman (1987) described an in vitro suppressor assay using responder cells from the lymph nodes of Lewis rats immunized sc. with acetylcholine receptors emulsified in complete Freund's adjuvant and suppressor cells from spleens of rats immunized i.p. with acetylcholine receptors absorbed on bentonite. Antibodies were determined after stimulation with acetylcholine receptors from cocultures of responder cells and putative suppressor cells treated previously with an immunosuppressant.

Arag and Blalock (1994) developed a method of altering B cell-mediated autoimmune diseases by induction of anti-idiotypic antibodies by immunization with complementary peptides. A peptide encoded by RNA complementary to RNA for the Torpedo acetylcholine receptor main immunogenic region, AChR 67–16, was tested in the Lewis rat model of experimental autoimmune myasthenia gravis.

Russell et al. (2012) reported on the testing of CK-2017357 (Tirasemtiv) in rat model of myasthenia gravis and showed as a troponin activator it improved muscle function in this model.

Oliveira et al. (2015) describe the role of CD73 in impaired neuromuscular transmission in the EMG model and further describe the potential role of adenosine in the pathophysiology on this neuromuscular disorder.

References and Further Reading

Arag S, Blalock JE (1994) Use of complementary peptides and their antibodies in B-cell-mediated autoimmune disease: prevention of autoimmune myasthenia gravis with a peptide vaccine. Immunomethods 5:130–135

Damjanovic M, Vidic-Dankovic B, Kosec D, Isakovic K (1993) Thymus changes in experimentally induced myasthenia gravis. Autoimmunity 15:201–207

Drachman DB, Adams RN, McIntosh K, Pestronk A (1985) Treatment of experimental myasthenia gravis with cyclosporin A. Clin Immunol Immunopathol 34:174–188

Harrison C (2012) Neuromuscular disorders: troponin activator improves muscle function. Nat Rev Drug Discov 11:272–273

Itoyama Y, Kira J, Fuji N, Goto I, Yamamoto N (1989) Increases in helper inducer T cells and activated T cells in HTLV-1 associated myelopathy. Ann Neurol 26:257–262

Lennon VA, Lambert EH, Leiby KR, Okarma TB, Talib S (1991) Recombinant human acetylcholine receptor α-subunit induces chronic experimental autoimmune myasthenia gravis. J Immunol 146:2245–2248

McIntosh KR, Drachman DB (1986) Induction of suppressor cells specific for AChR in experimental autoimmune myasthenia gravis. Science 232:401–403

McIntosh KR, Drachman DB (1987) Properties of suppressor cells induced to acetylcholine receptor using cyclosporin A. Ann N Y Acad Sci 505:628–638

Mrowka C, Thoenes GH, Langer KH, Bartlett RR (1994) Prevention of acute graft versus host disease (GVHD) in rats by the immunomodulating drug leflunomide. Ann Hematol 68:195–199

Norcross NL, Griffith IJ, Lettieri JA (1980) Measurement of acetylcholine receptors and antireceptor antibodies by ELISA. Muscle Nerve 3:345–349

Oliveira L, Correia A, Costa AC, Guerra-Gomes-S, Ferreirinha F, Magalhaes-Cardoso MT, Vilanova M, Correia-de-Sa P (2015) Deficits in endogenous adenosine formation by ecto-5′-nucleotidase/CD73 impair neuromuscular transmission and immune competence in experimental autoimmune myasthenia gravis. Mediat Inflamm 2015. Article ID 460610, 16 pp. doi:10.1155/2015/460610

Oosterhuis H (1981) Observations of the natural history of myasthenia gravis and effect of thymectomy. Ann N Y Acad Sci 377:678–682

Punga AR, Kaminski HJ, Richman DP, Benatar M (2015) How clinical trials of myasthenia gravis can inform pre-clinical drug development. Exp Neurol. doi: 10.1016/j.expneurol.2014.12.022

Russell AJ, Hartman JJ, Hinken AC, Muci AR, Kawas R, Driscoll L, Godinez G, Lee KH, Marquez D, Browne WF 4th, Chen MM, Clarke D, Collibee SE, Garard M, Hansen R, Jia Z, Lu PP, Rodriguez H, Saikali KG, Schaletzky J, Vijayakumar V, Albertus DL, Claflin DR, Morgans DJ, Morgan BP, Malik FI (2012) Activation of fast skeletal muscle troponin as a potential therapeutic approach for treating neuromuscular diseases. Nat Med 18:452–455

Tasgal J, Vaughan-Williams EM (1981) The effect of prolonged propranolol administration on myocardial transmural capillary density in young rabbits. J Physiol 315:353–367

Ulrichs K, Kaitschick J, Bartlett R, Müller-Ruchholtz W (1992) Suppression of natural xenophile antibodies with the novel immunosuppressive drug leflunomide. Transplant Proc 24:718–719

Weible ER (1963) Principles and methods for the morphometric study of the lung and other organs. Lab Invest 12:131–155

Williams JW, Xiao F, Foster P, Clardy C, McChesney L, Sankary H, Chong ASF (1994) Leflunomide in experimental transplantation. Control of rejection and alloantibody production, reversal of acute rejection, and interaction with cyclosporine. Transplantation 57:1223–1231

Glomerulonephritis Induced by Antibasement Membrane Antibody in Rats

Purpose and Rationale

Masugi nephritis and other nephritis models of immunological origin in rats have been used for evaluation of immunosuppressive activity (Heymann et al. 1959; Shibata et al. 1966; Ito et al. 1983; Thoenes et al. 1989; Ogawa et al. 1990, 1991).

Procedure

Preparation of Rabbit Antiserum Against Rat Glomerular Basement Membrane

Glomeruli are separated from the homogenate of rat renal cortex by successive use of three metal sieves (150-, 180-, and 200-mesh). The basement membrane fraction is obtained by centrifugation and ultrasonic disruption. It is then digested with trypsin, dialyzed, and lyophilized. The resultant substance is employed as antigen. An emulsion of 1 mg of the antigen in 0.2 ml saline with 0.2 ml of complete Freund's adjuvant is injected intracutaneously into white rabbits once a week for 6 weeks. One week later, production of the antibasement membrane antibody is confirmed in guinea pigs by the passive cutaneous anaphylaxis test. The blood is collected from the carotid artery, incubated at 56 °C for 30 min to inactivate components of the complement and stored at −20 °C until use.

Induction of Glomerulonephritis in Rats

Male Sprague–Dawley rats weighing about 300 g are injected with 0.5 ml of the rabbit antiserum via the tail vein. On the following day, they are further injected subcutaneously with an emulsion (0.25 ml) of physiological saline solution containing 5 mg of rabbit gamma globulin in an identical volume of complete Freund's adjuvant.

Treatment

The rat antibasement antibody is injected 5 days before the start of administration of the test compound. Before the first dose, urinary total protein is determined and rats with nephritis are so assigned as to provide almost equal distribution of severity of the disease per group. The test compounds are administered orally for 14 days. The urine is collected at 7 and 14 days of treatment. After 14 days, the animals are sacrificed, blood is collected, and the thymus and kidneys are removed. Histopathological and immunohistochemical studies are performed in kidney tissue.

Evaluation

Scores are given for **microscopic findings in the following:**

Glomeruli

- Cell proliferation in glomeruli
- PAS-positive granules in the epithelium of glomeruli
- Fibrin deposits in Bowman's space
- Adhesion to Bowman's capsule

Tubuli
- Hyaline cast
- Dilation of tubuli

Scores are also given for **immunofluorescence findings** for rat IgG, rat C3, and rabbit IgG.

Furthermore, total urinary protein, plasma total cholesterol, plasma fibrinogen, and thymus/body weight ratio are compared between drug-treated animals and controls by statistical means.

Modifications of the Method

Lan et al. (1995) investigated the pathogenic role of interleukin-1 in the progression of established rat crescentic glomerulonephritis by administration of the interleukin-1 receptor antagonist IL-1ra.

Giménez et al. (1987) and Thoenes et al. (1987) induced autoimmune tubulointerstitial nephritis in the Brown Norway rat by injection of bovine tubular basement membrane.

Development of a systemic T lymphocyte-dependent autoimmune syndrome in Brown Norway rats including glomerulonephritis with high proteinuria was induced with mercuric chloride by Baran et al. (1986), Aten et al. (1988), and Lillevang et al. (1992).

Kokui et al. (1992) induced nephrosis with proteinuria in rats by intraperitoneal injection of puromycin aminonucleoside.

Lundstrom et al. (1993) studied the Heymann nephritis antigenic complex using a rat yolk sac carcinoma cell line that expresses glycoprotein 330, the main antigen in this autoimmune disease.

Taylor et al. (2009) demonstrated a role for the purinergic P2X7 purinoreceptor in experimental glomerulonephritis showing that mice harboring a knockout for the receptor were renoprotective, further supported by a nonclinical intervention study with A-439079. Smith et al. (2010) investigated the role of spleen tyrosine kinase (SYK) in a rat model of glomerulonephritis with R788 (fostamatinib) and showed reduction of glomerular crescents and improvement in renal function establishing SYK as a target for potential future clinical investigation.

Suana et al. (2011) have shown that immunoliposomes carrying a low-dose mycophenolate mofetil cargo may prevent creatine increase and albuminuria in a model of experimental mesangial proliferative glomerulonephritis model in the rat.

D'Souza et al. (2013) developed a bicongenic rat model of experimental crescent glomerulonephritis to develop a system for investigating macrophage-dependent glomerulonephritis.

Recently Takakura et al. (2014) demonstrate an antiproliferative effect of the anti-inflammatory and antifibrotic agent pirfenidone in a rat model of basement membrane glomerulonephritis.

References and Further Reading

Aten J, Bosman CB, de Heer E, Hoedemaeker PJ, Weening JJ (1988) Cyclosporin A induces long-term unresponsiveness in mercuric chloride-induced autoimmune glomerulonephritis. Clin Exp Immunol 73:307–311

Baran D, Vendeville B, Vial MC, Cosson C, Bascou C, Teychenne P, Druet P (1986) Effect of cyclosporin A on mercury-induced autoimmune glomerulonephritis in the Brown Norway rat. Clin Nephrol 25(Suppl 1):S175–S180

Cattran DC (1988) Effect of cyclosporin on active Heymann nephritis. Nephron 48:142–148

D'Souza Z, McAdoo SP, Smith J, Pusey CD, Cook HT, Behmoaras J, Aitman TJ (2013) Experimental crescentic glomerulonephritis: a new bicongenic rat model. Dis Model Mech 6:1477–1486

Fujita M, Ilida H, Asaka M, Izumino K, Takata M, Sasayama S (1991) Effect of the immunosuppressive agent, cyclosporin, on experimental immune complex glomerulonephritis in rats. Nephron 57:210–215

Giménez A, Leyva-Cobian F, Fiero C, Rio M, Bricio T, Mampaso F (1987) Effect of cyclosporin A on autoimmune tubulointerstitial nephritis in the brown Norway rat. Clin Exp Immunol 69:550–556

Grönhagen-Riska C, von Willebrand E, Tikkanen T, Honkanen E, Miettinen A, Holthöfer H, Törnroth T (1990) The effect of cyclosporin A on the interstitial mononuclear cell infiltration and the induction of Heymann's nephritis. Clin Exp Immunol 79:266–272

Heymann W, Hackel DB, Harwood S, Wilson SGF, Hunter JLP (1959) Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspension. Proc Soc Exp Biol Med 100:660–664

Ito M, Yamada H, Okamoto K, Suzuki Y (1983) Crescentic type nephritis induced by anti-glomerular basement membrane (GMB) serum in rats. Jpn J Pharmacol 33:1145–1154

Kokui K, Yoshikawa N, Nakamura H, Itoh H (1992) Cyclosporin reduces proteinuria in rats with aminonucleoside nephrosis. J Pathol 166:297–301

Lan HY, Nikolic-Paterson DJ, Mu W, Vannice JL, Atkins RC (1995) Interleukin-1 receptor antagonist halts the progression of established crescentic glomerulonephritis in the rat. Kidney Int 47:1303–1309

Lillevang ST, Rosenkvist J, Andersen CB, Larsen S, Kemp E, Kristensen T (1992) Single and combined effects of the vitamin D analogue KH1060 and cyclosporin A on mercuric-chloride-induced autoimmune disease in the BN rat. Clin Exp Immunol 88:301–306

Lundstrom M, Orlando RA, Saedi MS, Woodward L, Kurihara H, Farquhar MG (1993) Immunocytochemical and biochemical characterization of the Heymann nephritis antigenic complex in rat L2 yolk sac cells. Am J Pathol 143:1423–1435

Ogawa T, Inazu M, Gotoh K, Hayashi S (1990) Effects of leflunomide on glomerulonephritis induced by antibasement membrane antibody in rats. Agents Actions 31:321–328

Ogawa T, Inazu M, Gotoh K, Inoue T, Hayashi S (1991) Therapeutic effects of leflunomide, a new antirheumatic drug, on glomerulonephritis induced by the antibasement antibody in rats. Clin Immunol Immunopathol 61:103–118

Reynolds J, Cashman SJ, Evans DJ, Pusey CD (1991) Cyclosporin A in the prevention and treatment of experimental autoimmune glomerulonephritis in the brown Norway rat. Clin Exp Immunol 85:28–32

Schorlemmer HU, Dickneite G (1992) Preclinical studies with 15-deoxyspergualin in various animal models for autoimmune diseases. Ann N Y Acad Sci 685:155–174

Shibata S, Nagasawa T, Takuma T, Naruse T, Miyakawa Y (1966) Isolation and properties of the soluble antigen specific for the production of nephrotoxic glomerulonephritis. I. Immunopathological demonstration of the complete antigenicity of the soluble antigen. Jpn J Exp Med 36:127–143

Shih W, Hines WH, Neilson EG (1988) Effects of cyclosporin A on the development of immune-mediated interstitial nephritis. Kidney Int 33:1113–1118

Smith J, McDaid JP, Bhangal G, Chawanasuntorapoj R, Masuda ES, Cook HT, Pusey CD, Tam FWK (2010) A spleen tyrosine kinase inhibitor reduces the severity of established glomerulonephritis. J Am Soc Nephrol 21:231–236

Suana AJ, Tuffin G, Frey BM, Knudsen L, Muhlfeld C, Rodder S, Marti H-P (2011) Single application of low dose mycophenolate mofetil-OX7- immunoliposomes ameliorates experimental mesangial proliferative glomerulonephritis. J Pharmacol Exp Ther 337:411–422

Takakura K, Mizukami K, Mitori H, Noto T, Tomura Y (2014) Antiproteinuric effect of pirfenidone in a rat model of anti-glomerular basement membrane glomerulonephritis. Eur J Pharmacol 737:106–116

Taylor SRJ, Turner CM, Elliott JI, McDaid J, Hewitt R, Smith J, Pickering MC, Whitehouse DL, Cook HT, Burnstock G, Pusey CD, Unwin RJ, Tam FWK (2009) $P2X_7$ deficiency attenuates renal injury in experimental glomerulonephritis. J Am Soc Nephrol 20:1275–1281

Thoenes GH, Umscheid T, Sitter T, Langer KH (1987) Cyclosporin A inhibits autoimmune experimental tubulointerstitial nephritis. Immunol Lett 15:301–306

Thoenes GH, Sitter T, Langer KH, Bartlett RR, Schleyerbach R (1989) Leflunomide (HWA 486) inhibits experimental autoimmune tubulointerstitial nephritis in rats. Int J Immunopharmacol 11:921–929

Tipping PG, Holdsworth SR (1985) Effect of cyclosporin A on antibody-induced experimental glomerulonephritis. Nephron 40:201–205

Tipping PG, Neale TJ, Holdsworth SR (1985) T lymphocyte participation in antibody-induced experimental glomerulonephritis. Kidney Int 27:530–537

Wilson CB (1981) Nephritogenic antibody mechanisms involving antigens within the glomerulus. Immunol Rev 55:257–297

Wood A, Adu D, Birtwistle RJ, Brewer DB, Michael J (1988) Cyclosporin A and anti-glomerular basement membrane antibody glomerulonephritis in rats. Br J Pathol 69:189–193

Inhibition of Allogenic Transplant Rejection

Purpose and Rationale

Transplantation of allogenic organs to recipients results in rejection of the transplants (Sanchez-Fueyo and Strom 2011). This effect can be suppressed or delayed by immunosuppressive agents, and the role of B cells has been investigated in animal models suggesting a role in mechanisms of transplant tolerance (Chesneau et al. 2013). Various organs are used for allogenic transplantation in animal experiments, such as skin pieces (Schorlemmer et al. 1993), kidney (Lee 1967; Küchle et al. 1991), rat heart, rat small intestine (Xiao et al. 1994; Zhang et al. 2014), and corneal buttons (Coupland et al. 1994). The immunosuppressive activity can be evaluated either by using a major histocompatibility complex variant strain combination or a strong allogenic system, and the advances and limitations of murine models have been recently described (Schroeder and DiPersio 2011).

Procedure

For **skin transplantation** male animals of inbred strains of Fischer (F334), Lewis (LEW), Brown Norway (BN), and Dark Agouti (DA) rats are used. Rat tail skin (donor) is cut into square pieces of 0.5–1.0 cm and transplanted to the tails of recipient rats. Rejection is defined as the day when the skin graft is of red-brown color and hard consistency. As strain combination with a major histocompatibility variant, transplantation from LEW to F334 is performed. Using a strong allogenic system, the high responder DA to LEW donor-recipient combination is used. The immunosuppressive agents, e.g., cyclosporine or leflunomide, are given orally up to 20 days. Ten animals are used for each group.

Evaluation

The mean values of rejection time of treated groups are compared statistically with vehicle-treated controls using Student's *t*-test or the Mann–Whitney *U*-test.

Modifications of the Method

Schorlemmer and Kurrle (1997) used Lewis (LEW, Rtl*l) rats as receivers and Balb/c mice as donors in a xenotransplantation model of mouse-to-rat skin grafts. Rejection was defined as the day when the skin graft turned red-brown and became hard. For quantification of xenospecific IgM and IgG antibody titers, the test sera (dilution 1:10) were incubated with 1×10^6 purified T cells (by sheep anti-mouse Dynabeads, Deutsche Dynal GmbH, Hamburg, Germany) from Balb/c donor spleens for 30 min at 4 °C. The cells were washed three times with phosphate-buffered saline (pH 7.2) and then stained for IgG or IgM xenoantibodies; 50 µl of FITC-conjugated goat antibodies, specific for the Fc-portion of rat IgG or specific for the µ-chain of rat IgM, was added. After 30 min at 4 °C, the cells were washed twice and analyzed by flow cytometry.

Techniques for transplantation of several organs have been elaborated.

For **kidney transplantation**, male rats, 5–7 months of age, are used as donors and recipients for the orthotopic right kidney transplantation as described by Lee (1967) with a modification of ureter–ureter anastomosis (Thoenes et al. 1974). Because bilateral nephrectomy is performed at transplantation, animal survival is dependent upon the allograft's function. All rats that do not excrete urine on the first postoperative day are excluded from further studies. As a control concerning long survival, syngenically transplanted rats are maintained up to 300 days.

Engelbrecht et al. (1992) described a new rapid technique for renal transplantation in the rat. The method combines a special sleeve anastomotic

technique for the renal artery, conventional end-to-end anastomosis of the renal vein, and implantation of the ureter into the bladder.

A porcine renal transplant model has been used by Almond et al. (1992).

Peters et al. (1993) reviewed the therapeutic potential of tacrolimus in renal and hepatic transplantation.

For studying **heart transplantation**, heterotopic implantation of hearts from BN to LEW rats is performed (Williams et al. 1993). The diagnosis of rejection is established once the palpable cardiac allograft impulse ceases. Further studies with rat cardiac allografts have been performed by Hancock et al. (1990). The Fischer 344 rat (donor)/Long Evans rat (recipient) combination was used by Kahn et al. (1991). Walpoth et al. (1993) used magnetic resonance spectroscopy for assessing myocardial rejection in the transplanted rat heart.

Shiraishi et al. (1995) evaluated the effectiveness of the interleukin-1 receptor antagonist IL-1ra in the immune and inflammatory responses to rat heart allografts.

Cardiac transplantation between inbred rat strains that differ for weak histocompatibility antigens is associated with the development of arteriosclerosis in arteries of the donor graft myocardium (Cramer et al. 1990; Adams et al. 1992).

A heterotopic rat **heart transplant model** and the influence of infection were described by Kobayashi et al. (1993).

The **hamster to rat cardiac xenograft** model has been used by several authors (de Masi et al. 1990; Steinbrüchel et al. 1991; van den Bogaerde et al. 1991; Woo et al. 1993; Fujino et al. 1994; Schuurman et al. 1994). The hearts from Syrian hamsters were implanted heterotopically in male Lewis rats, with anastomoses between the infrarenal abdominal aorta and inferior vena cava of the recipient and the donor aorta and right pulmonary artery, respectively.

Primate cardiac xenografts were performed by McManus et al. (1993) using cynomolgus monkeys (*Macaca fascicularis*) as donors and baboons (*Papio anubis*) as recipients.

Chronic rejection of rat **aortic allograft** was studied by Mennander et al. (1991). Administration of cyclosporine induced accelerated allograft arteriosclerosis.

Heterotopic transplantation of small intestine has been performed from BN to LEW rats. The mesenteric venous drainage is reconstructed either via the vena cava or the portal vein (Xiao et al. 1994). An isolated Thiry–Vella loop was prepared by Xia and Kirkman (1990). Kellnar et al. (1990) described allogenic transplantation of fetal rat intestine with anastomosis to the normal bowel of the host. Langrehr et al. (1991) investigated under which circumstances graft-versus-host disease occurs following fully allogenic small bowel transplantation in the rat. Kirsch et al. (1991) studied the extent to which intestinal transplants in rats undergo functional and morphologic compensation.

Liver transplantation procedure has been described by Svensson et al. (1995), allowing measurement of bile secretion.

Orthotopic left **lung transplantation** was performed in inbred rats by Katayama et al. (1991).

Tracheal allografts were implanted into the abdomen of recipient rats (Davreux et al. 1993).

In vivo electrophysiology of rat **peripheral nerve transplants** was studied by Yu et al. (1990). A sciatic-tibial nerve graft was harvested from the donor rat between the sciatic notch and the ankle. In the recipient, the tibial nerve and the sural nerve were resected. The nerve graft was placed along the natural course of the native tibial nerve. Nerve repair was performed using standard end-to-end epineural microsuture technique.

A model of neurovascularized rectus femoris **muscle transplantation** in rats was established by Muramatsu et al. (1994).

The orthotopic **transplantation of vascularized skeletal allografts** (rat distal femur and surrounding muscular cuff) has been described by Lee et al. (1995).

Long-term survival of **limb allografts** in rats was studied by Kuroki et al. (1991). The donor and recipient limbs were prepared simultaneously by amputation at mid-femur. The donor limb was

fixed orthotopically by Kirschner wire. The donor and recipient femoral arteries, veins, and sciatic nerves were anastomosed using a microsurgical technique.

For **cornea transplantation**, Brown Norway rats ($RT1^{1\times n}$) serve as donors and Lewis rats ($RT1^{1}$) as recipients (Coupland et al. 1994). Both the donor and recipient rats are anesthetized with xylazine hydrochloride and ketamine hydrochloride. Twenty min prior to surgery, the recipient rats also receive 0.5 mg/kg atropine sc. and phenylephrine hydrochloride 5 % eyedrops. Under sterile conditions and using an operation microscope, two donor corneal buttons (3.5 mm) are harvested from the donor rat using a trephine and curved Castroviejo scissors. The donor animals are then sacrificed by ether inhalation. The left eyes of the recipient rats are prepared by removing a central 3.0-mm button using a trephine and curved Castroviejo scissors. A drop of sterile methylcellulose (1 %) is placed over the 3.0-mm corneal opening before the donor cornea is fixed with 10 interrupted sutures. The anterior chamber is not reestablished following surgery. Prior to closure of the eyelids with three or four interrupted sutures, Polyspectran eyelid gel is placed over the operated eye. Forty-eight hours following surgery, the eyelid sutures are removed, allowing for the first time assessment of the cornea on the slit-lamp microscope. Slit-lamp evaluations are performed every 2–3 days under i.m. anesthesia with ketamine, with assessment of the cornea by scoring graft opacity, edema, and vascularization.

Recently the role of indoleamine 2,3-dioxygenase as an immunomodulator has been reviewed in models of allogenic pancreatic islet and skin transplantation (Gill et al. 2013).

References and Further Reading

Adams DH, Tiney NL, Collins JJ, Karnovsky MJ (1992) Experimental graft arteriosclerosis. Transplantation 53:1115–1119

Almond PS, Moss A, Nakhleh R, Melin M, Chen S, Salazar A, Shirabe K, Matas A (1992) Rapamycin in a renal transplant model. Ann N Y Acad Sci 685:121–122

Bartlett RR, Dimitrijevic M, Mattar T, Zielinski T, Germann T, Rüde E, Thoenes GH, Küchle CCA, Schorlemmer HU, Bremer E, Finnegan A, Schleyerbach R (1991) Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of autoimmune disorders and reactions leading to transplantation rejection. Agents Actions 32:11–21

Chesneau M, Michel L, Degauque N, Brouard S (2013) Regulatory B cells and tolerance in transplantation: from animal models to human. Front Immunol 4:1–8

Coupland SE, Klebe S, Karow AC, Krause L, Kruse H, Bartlett RR, Hoffmann F (1994) Leflunomide therapy following penetrating keratoplasty in the rat. Graefe's Arch Clin Exp Ophthalmol 232:622–627

Cramer DV, Chapman FA, Wu GD, Harnaha JB, Qian S, Makowka L (1990) Cardiac transplantation in the rat. Transplantation 50:554–558

Davreux CJ, Chu NH, Waddell TK, Mayer E, Patterson GA (1993) Improved tracheal allograft viability in immunosuppressed rats. Ann Thorac Surg 55:131–134

de Masi R, Alqaisi M, Araneda D, Nifong W, Thomas J, Gross U, Swanson M, Thomas F (1990) Reevaluation of total-lymphoid irradiation and cyclosporine therapy in the Syrian hamster-to-Lewis rat cardiac xenograft model. Transplantation 49:639–641

Engelbrecht G, Kahn D, Duminy F, Hickman R (1992) New rapid technique for renal transplantation in the rat. Microsurgery 13:340–344

Fujino Y, Kawamura T, Hullett DA, Sollinger HW (1994) Evaluation of cyclosporine, mycophenolate mofetil, and brequinar sodium combination therapy on hamster-to-rat cardiac xenotransplantation. Transplantation 57:41–46

Gill P, Jalili R, Ghahary A (2013) Immunomodulatory role of indoleamine 2,3-dioxygenase in allogeneic islet and skin transplantation. Res Immunol Int J 2013. Article ID 235635. doi:10.5171/2013.235635

Hancock WW, diStefano R, Braun P, Schweizer RT, Tilney NL, Kupiec-Weglinski JW (1990) Cyclosporin and anti-interleukin 2 receptor monoclonal antibody therapy suppress accelerated rejection of rat cardiac allografts through different effector mechanisms. Transplantation 49:416–421

Kahn DR, Forrest DE, Otto DA (1991) Prolonged survival of rat cardiac allografts by donor pretreatment with methotrexate. Transplantation 51:697–700

Katayama Y, Yada I, Namikawa S, Kusagawa M (1991) Immunosuppressive effects of FK 506 in rat lung transplantation. Transplant Proc 23:3300–3301

Kellnar S, Herkomer C, Bae S, Schumacher U (1990) Allogenic transplantation of fetal rat intestine: anastomosis to the normal bowel of the host. J Pediatr Surg 25:415–417

Kirsch AJ, Kirsch SS, Kimura K, LaRosa CA, Jaffe BM (1991) The adaptive ability of transplanted rat small intestine. Surgery 109:779–787

Kobayashi J, Mavroudis C, Crawford SE, Zales VR, Backer CL (1993) A new rat infection-heart transplant model: effect of infection on graft survival studies. J Heart Lung Transplant 12:659–664

Küchle CCA, Thoenes GH, Langer KH, Schorlemmer HU, Bartlett RR, Schleyerbach R (1991) Prevention of kidney and skin graft rejection in rats by leflunomide, a new immunomodulating agent. Transplant Proc 23:1083–1086

Kuroki H, Ishida O, Daisaku H, Fukuhara K, Hatano E, Murakami T, Ikuta Y, Matsumoto AK, Akiyama M (1991) Morphological and immunological analysis of rats with long-term-surviving limb allografts induced by a short course of FK 506 or cyclosporine. Transplant Proc 23:516–520

Langrehr JM, Hoffman RA, Banner B, Stangl MJ, Monyhan H, Le KKW, Schraut WH (1991) Induction of graft-versus-host disease and rejection by sensitized small bowel allografts. Transplantation 52:399–405

Lee S (1967) An improved technique of renal transplantation in the rat. Surgery 61:771

Lee WP, Pan YC, Kesmarky S, Randolph MA, Fiala TS, Amarante MTJ, Weiland AJ, Yaremchuk MJ (1995) Experimental orthotopic transplantation of vascularized skeletal allografts: functional assessment and long-term survival. Plast Reconstr Surg 95:336–353

McManus RP, O'Hair DP, Komorowski R, Scott JP (1993) Immunosuppressant combinations in primate cardiac xenografts. Ann N Y Acad Sci 969:281–284

Mennander A, Tiisala S, Paavonen T, Halttunen J, Häyry P (1991) Chronic rejection of rat aortic allograft. II. Administration of cyclosporin induces accelerated allograft arteriosclerosis. Transpl Int 4:173–179

Muramatsu K, Doi K, Kawai S (1994) The outcome of neurovascularized allogenic muscle transplantation under immunosuppression with cyclosporine. J Reconstr Microsurg 10:77–81

Murase N, Demetris AJ, Woo J, Tanabe M, Furuya T, Todo S, Strazl TE (1993) Graft-versus-host disease after Brown Norway-to-Lewis and Lewis-to-Brown Norway rat intestinal transplantation under FK 506. Transplantation 55:1–7

Nemoto K, Sugawara Y, Mae T, Hayashi M, Abe F, Fujii A, Takeuchi T (1992) Therapeutic activity of deoxyspergualin in comparison with cyclosporin A, and its combined use with cyclosporin A and prednisolone in highly allogeneic skin transplantation in the rat. Agents Actions 36:306–311

Peters DH, Fitton A, Plosker GL, Faulds D (1993) Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drug 46:746–794

Sanchez-Fueyo A, Strom TB (2011) Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. Gastroenterology 140:51–64

Schorlemmer HU, Kurrle R (1997) Synergistic activity of malononitrilamides with cyclosporine to control and reverse xenograft rejection. Int J Tissue React 19:149–156

Schorlemmer HU, Seiler FR, Bartlett RR (1993) Prolongation of allogenetic transplanted skin grafts and induction of tolerance by leflunomide, a new immunosuppressive isoxazol derivative. Transplant Proc 25:763–767

Schroeder MA, DiPersio JF (2011) Mouse models of graft-versus-host disease: advances and limitations. Dis Model Mech 4:318–333

Schuurman HJ, Joergensen J, Kuipers H, Meerloo T, Lardelli P, Hiestand P, White DH, Schreier MH (1994) Vascular transplantation of Syrian hamster heart into Lewis rat: effect of brequinar, cyclosporine, cobra venom factor, and splenectomy. Transplant Proc 26:1217–1219

Shaffer D, Muanza T, Blakely ML, Simpson MA, Monaco AP (1993) Prevention of graft-versus-host-disease by RS-61443 in two different rodent models. Transplantation 55:221–223

Shiraishi M, Csete M, Yasunaga C, McDiarmid SV, Vannice JL, Busuttil RW, Shaked A (1995) The inhibitor cytokine interleukin-1 receptor antagonist synergistically augments cyclosporine immunosuppression in a rat cardiac allograft model. J Surg Res 58:465–470

Steinbrüchel DA, Madsen HH, Nielsen B, Kemp E, Larsen S, Koch C (1991) The effect of combined treatment with total lymphoid irradiation, cyclosporin A, and anti-CD4 monoclonal antibodies in a hamster-to-rat heart transplantation model. Transplant Proc 23:579–580

Svensson G, Holmberg SB, Friman S (1995) Influence of liver transplantation and cyclosporin on bile secretion – an experimental study in the rat. Transpl Int 8:27–34

Thoenes GH, Urban G, Doering I (1974) Kidney transplantation between congenic versus standard inbred strains of rats. I. The significance of H-1 and non-H-I gene differences. Immunogenet 3:239–253

Ulrichs K, Kaitschick J, Bartlett R, Müller-Ruchholtz W (1992) Suppression of natural xenophile antibodies with the novel immunomodulating drug leflunomide. Transplant Proc 24:718–719

van den Bogaerde J, Aspinall R, Wang MW, Cary N, Lim S, Wright L, White D (1991) Induction of long-term survival of hamster heart xenografts in rats. Transplantation 52:15–20

Walpoth BH, Tschopp A, Lazeyras F, Galdikas J, Tschudi J, Altermatt H, Schaffner T, Aue WP, Althaus U (1993) Magnetic resonance spectroscopy for assessing myocardial rejection in the transplanted rat heart. J Heart Lung Transplant 12:271–282

Williams JW, Xiao F, Foster P, Chong A, Sharma S, Bartlett RR, Sankary HN (1993) Immunosuppressive effects of leflunomide in a cardiac allograft model. Transplant Proc 25:745–746

Williams JW, Xiao F, Foster P, Clardy C, McChesney L, Sankary H, Chong ASF (1994) Leflunomide in experimental transplantation. Control of rejection and alloantibody production, reversal of acute rejection, and interaction with cyclosporine. Transplantation 57:1223–1231

Woo J, Valdivia LA, Pan F, Celli S, Fung JJ, Thomson AW (1993) Cytidine potentiates the inhibitory effect of brequinar sodium on xeno-MLR, antibody production, and concordant hamster to rat cardiac xenograft survival. Ann N Y Acad Sci 969:227–234

Xia W, Kirkman RL (1990) Immune function in transplanted small intestine. Transplantation 49:277–280

Xiao F, Chong ASF, Bartlett RR, Williams JW (1994) Leflunomide: a promising immunosuppressant in transplantation. In: Thomson AW, Starzl TE (eds) Immunosuppressive drugs. Edward Arnold, London/Boston/Melbourne, pp 203–212

Yu LT, England J, Sumner A, Larossa D, Hickey WF (1990) Electrophysiologic evaluation of peripheral nerve regeneration through allografts immunosuppressed with cyclosporin. J Reconstr Microsurg 6:317–323

Zhang X, Liu Y, Zhang G, Shi J, Zhang X, Zheng X, Jiang AT, Zhang Z-X, Johnston N, Siu KS, Chen R, Lian D, Koos D, Quan D, Min W-P (2014) Synergic silencing of costimulatory molecules prevents cardiac allograft rejection. J Trans Med 12:142. doi:10.1186/1479-5876-12-142

References and Further Reading

Inhibition Of Histamine Release from Mast Cells

Ali H, Brøgger Christensen S, Foreman JC, Pearce FL, Piotrowski W, Thastrup O (1985) The ability of thapsigargin and thapsigargicin to activate cells involved in the inflammatory response. Br J Pharmacol 85:705–712

Bartlett RR, Dimitrijevic M, Mattar T, Zielinski T, Germann T, Rüde E, Thoenes GH, Küchle CCA, Schorlemmer HU, Bremer E, Finnegan A, Schleyerbach R (1991) Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of auto-immune disorders and reactions leading to transplantation rejection. Agents Actions 32:11–21

Broide D, Marquardt D, Wasserman S (1986) Effect of nedocromil sodium and sodium cromoglycate on connective tissue and bone marrow derived mast cells: acute and chronic studies. Eur J Respir Dis 69(Suppl 147):196–198

Church MK, Young KD (1983) The characteristics of inhibition of histamine release from human lung fragments by sodium cromoglycate, salbutamol and chlorpromazine. Br J Pharmacol 78:671–679

Eady RP (1986) The pharmacology of nedocromil sodium. Eur J Respir Dis 69(Suppl 147):112–119

Flint KC, Leung KBP, Oearce FL, Hudspith BN, Brostoff J, Johnson N (1985) Human mast cells recovered by bronchoalveolar lavage: their morphology, histamine release and the effects of disodium cromoglycate. Clin Sci 68:427–432

Johnson HG, Bach MK (1975) Prevention of calcium ionophore-induced release of histamine in rat mast cells by disodium cromoglycate. J Immunol 114:514–516

Johnston RB, Godzik CA, Cohn ZA (1978) Increased superoxide anion production by immunologically activated and chemically elicited macrophages. J Exp Med 148:115–127

Kase K, Hua J, Yokoi H, Ikeda K, Nagaoka I (2009) Inhibitory action of roxithromycin on histamine release and prostaglandin D_2 production from β-defensin 2-stimulated mast cells. Int J Mol Med 23:337–340

Kay AB, Walsh GM, Moqbel R, MacDonald AJ, Nagakura T, Carroll MP, Richerson HB (1987) Disodium cromoglycate inhibits activation of human inflammatory cells in vitro. J Allergy Clin Immunol 80:1–8

Lavens SE, Proud D, Warner JA (1993) A sensitive colorimetric assay for the release of tryptase from human lung mast cells in vitro. J Immunol Methods 166:93–102

Lawrence ID, Warner JA, Cohan VL, Lichtenstein LM, Kagey-Sobotka A, Vavrek JR, Stewart JM, Proud D (1989) Induction of histamine release from human skin mast cells by bradykinin analogs. Biochem Pharmacol 38:227–233

Orr TSC, Cox JSG (1969) Disodium cromoglycate, an inhibitor of mast cell degranulation and histamine release induced by phospholipase A. Nature 223:197–198

Orr TSC, Hall DE, Gwilliam JM, Cox JSG (1971) The effect of sodium cromoglycate on the release of histamine and degranulation of rat mast cells induced by compound 48/80. Life Sci 10:805–812

Peretti M, Nuti S, Parente L (1990) Investigation of rat mast cell degranulation using flow cytometry. J Pharmacol Methods 23:187–194

Riley PA, Mather ME, Keogh RW, Eady RP (1987) Activity of nedocromil sodium in mast-cell-dependent reactions in the rat. Int Arch Allergy Appl Immunol 82:108–110

Siriganian RP (1976) Histamine release and assay methods for the study of human allergy. In: Rose NR, Friedman H (eds) Manual of clinical immunology. American Society of Microbiology, Washington, pp 603–615

Skolfitsch G, Saria A, Holzer P, Lembeck F (1981) Histamine in tissue: determination by high-performance liquid chromatography PLC condensation with *o*-phthalaldehyde. J Chromatogr 226:53–59

Wells E, Jackson CG, Harper ST, Mann J, Eady RP (1986) Characterization of primate bronchoalveolar mast cells. II. Inhibition of histamine, LTC_4, and PGD_2 release from primate bronchoalveolar mast cells and a comparison with rat peritoneal mast cells. J Immunol 137:3941–3945

Williams PD, Laska DA, Shetler TJ, McGrath JP, White SL, Hoover DM (1991) Vancomycin-induced release of histamine from rat peritoneal mast cells and a rat basophil cell line (RBL-1). Agents Actions 32:217–223

Yazid S, Sinniah A, Solito E, Calder V, Flower RJ (2013) Anti-allergic cromones inhibit histamine and eicosanoid release from activated human and murine mast cells by releasing annexin A1. PLoS One 8:e58963

Mitogen Induced Lymphocyte Proliferation

Bartlett RR (1986) Immunopharmacological profile of HWA 486, a novel isoxazol derivative-II. in vivo immunomodulating effects differ from those of cyclophosphamide, prednisolone, or cyclosporin A. Int J Immunopharmacol 8:199–204

Delgado IF, Paumgartten FJR (2014) Effects of *Euphorbia milii* latex on mitogen-induced lymphocyte proliferation. Rev Bras Pl Med Camp 16:107–111

di Padova FE (1989) Pharmacology of cyclosporine (Sandimmune) V. Pharmacological effects on immune function: in vitro studies. Pharmacol Rev 41:373–405

Elves MW (1972) The lymphocytes, Chap 7. In: In vitro lymphocyte transformation and antibody formation,

2nd edn., Year Book Medical Publishers, Chicago, pp 381–457
Keller JM, McClellan-Green PD, Lee AM, Arendt MD, Maier PP, Segars AL, Whitaker JD, Keil DE, Peden-Adams MM (2005) Mitogen-induced lymphocyte proliferation in logger head sea turtles: comparison of methods and effects of gender, plasma testosterone concentration and body condition on immunity. Vet Immunol Immunopathol 103:269–281
Nikitin PA, Price AM, McFadden K, Yan CM, Luftig MA (2014) Mitogen-induced B-cell proliferation activates Chk2-dependent G1/S cell cycle arrest. PLoS One 9: e87299
Sensi M, di Mario U, Pozzilli P (1984) Lymphocyte populations. Evaluation of T and B populations, T cell subpopulations and K cells. In: Larner J, Pohl SL (eds) Methods in diabetes research, vol I, Laboratory methods, Part B. Wiley, New York, pp 77–97
Yamamura M, Nikbin B, Hobbs JR (1976) Standardisation of the mixed lymphocyte reaction. J Immunol Methods 10:367–378
Zan-Bar I (1983) Modulation of B and T cell subsets in mice treated with fractionated total lymphoid irradiation. I. Blockade of differentiating B cell pathways. Eur J Immunol 13:35–40

Inhibition of T Cell Proliferation

Chong ASF, Finnegan A, Jiang XL, Gebel H, Sankary HN, Foster P, Williams JW (1993a) Leflunomide, a novel immunosuppressive agent. Transplantation 55: 1361–1366
Chong ASF, Gebel H, Finnegan A, Petraitis EE, Jiang XL, Sankary HN, Foster P, Williams JW (1993b) Leflunomide, a novel immunomodulatory agent: in vitro analyses of the mechanism of immunosuppression. Transplant Proc 25:747–749
Clipstone NA, Crabtree GR (1993) Calcineurin is a key signaling enzyme in T lymphocyte activation and the target of the immunosuppressive drugs cyclosporin A and FK506. Ann N Y Acad Sci 696:20–30
Dayton JS, Turka LA, Thompson CB, Mitchell BS (1992) Comparison of the effects of mizoribine with those of azathioprine, 6-mercaptopurine, and mycophenolic acid on T lymphocyte proliferation and purine ribonucleotide metabolism. Mol Pharmacol 41:671–676
di Padova FE (1989) Pharmacology of cyclosporine (Sandimmune) V. Pharmacological effects on immune function: in vitro studies. Pharmacol Rev 41:373–405
Frouin H, Menard L, Measures L, Brousseau P, Fournier M (2010) T lymphocyte-proliferative responses of a grey seal (*Halichoerus grypus*) exposed to heavy metals and PCBs in vitro. Aquat Mamm 36:365–371
Huber S, Hoffmann R, Muskens F, Voehringer D (2010) Alternatively activated macrophages inhibit T-cell proliferation by stat6-dependent expression of PD-L2. Blood 116:3311–3320
Klein AB, Witonsky SG, Ahmed SA, Holladay SD, Gogal RM Jr, Link L, Reilly CM (2006) Impact of different cell isolation techniques on lymphocyte viability and function. J Immunoassay Immunochem 27:61–76
Marshall HD, Urban SL, Welsh RM (2011) Virus-induced transient immune suppression and the inhibition of T cell proliferation by type I interferon. J Virol 85:5929–5939
Patsoukis N, Sari D, Boussiotis VA (2012) PD-1 inhibits T cell proliferation by upregulating p27 and p15 and suppressing cdc-25A. Cell Cycle 11:1–5
Morishima C, Shuhart MC, Wang CC, Paschal DM, Apodaca MC, Liu Y, Sloan DD, Graf TN, Oberlies NH, Lee DY-W, Jerome KR, Polyak SJ (2010) Silymarin inhibits in vitro T-cell proliferation and cytokine production in hepatitis C virus infection. Gastroenterology 138:671–681
Yamamura M, Nikbin B, Hobbs JR (1976) Standardisation of the mixed lymphocyte reaction. J Immunol Methods 10:367–378
Zielinski T, Müller HJ, Bartlett RR (1993) Effects of leflunomide (HWA 486) on expression of lymphocyte activation markers. Agents Actions 38(Spec Conf Issue):C80–C83
Zielinski T, Herrmann M, Müller HJ, Riedel N, Bartlett RR (1994) The influence of leflunomide on cell cycle, IL-2-receptor (IL-2-R) and its gene expression. Agents Actions 41(Spec Conf Issue):C204–C205
Zinocker S, Vaage JT (2012) Rat mesenchymal stromal cells inhibit T cell proliferation but not cytokine production through inducible nitric oxide synthase. Front Immunol 3:1–13

Chemiluminescence in Macrophages

Bartlett RR (1986) Immunopharmacological profile of HWA 486, a novel isoxazol derivative-II. in vivo immunomodulating effects differ from those of cyclophosphamide, prednisolone, or cyclosporin A. Int J Immunopharmacol 8:199–204
Bird J, Giroud JP (1985) An appraisal of the technique of polymorphonuclear leukocyte chemiluminescence as a means to detect compounds with antiinflammatory activity. J Pharmacol Methods 14:305–312
Johnson RB Jr, Codzik CA, Cohn ZA (1978) Increased superoxide anion production by immunologically activated and chemically elicited macrophages. J Exp Med 148:115–120
Kurosawa M, Hanawa K, Kobayashi S, Nakano M (1990) Inhibitory effects of azelastine on superoxide anion generation from activated inflammatory cells measured by a simple chemiluminescence method. Arzneim Forsch/Drug Res 40:767–770
Merétey K, Boehm U, Falus A (1983) Chemiluminescence response of human blood mononuclear cells to PAH and histamine. Agents Actions 13:237–240

Seeds MC, Parce JW, Szeijda P, Bass DA (1985) Independent stimulation of membrane potential changes and the oxidative metabolic burst in polymorphonuclear leukocytes. Blood 65:233–240

Selvaraj R, Sbarra AJ, Thomas GB, Cetrulo CL, Mitchell GW (1982) A microtechnique for studying chemiluminescence response of phagocytes using whole blood and its application to the evaluation of phagocytes in pregnancy. J Reticuloendothel Soc 31:3–16

Szliszka E, Mertas A, Czuba ZP, Krol W (2013) Inhibition of inflammatory response by Artepillin C in activated RAW264.7 macrophages. Evid Based Complement Alternat Med. doi: 10.1155/2013/735176

Traykov T, Hadjimitova V, Golivsky P, Ribarov S (1997) Effect of phenothiazines on activated macrophage-induced luminal-dependent chemiluminescence. Gen Physiol Biophys 16:3–14

Van Dyke K, Patel S, Vallyathan V (2003) Lucigenin chemiluminescence assay as an adjunctive tool for assessment of various stages of inflammation: a study of quiescent inflammatory cells. J Biosci 28:115–119

Weidemann MJ, Smith R, Heaney T, Alaudeen S (1980) On the mechanism of the generation of chemiluminescence by macrophages. Behring Inst Mitt 65:42–54

Weinberg JB, Misokonis MA (1983) Phorbol diester-induced H_2O_2 production by peritoneal macrophages. Cell Immunol 80:405–415

PFC (Plaque Forming Colony) Test In Vitro

Bartlett RR (1986) Immunopharmacological profile of HWA 486, a novel isoxazol derivative – II. In vivo immunomodulating effects differ from those of cyclophosphamide, prednisolone, or cyclosporin A. Int J Immunopharmacol 8:199–204

Borel JF, Feurer C, Gubler HU, Stähelin H (1976) Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions 6:468–475

Cunningham AJ, Szenberg A (1968) Further improvements in the plaque technique for detecting single antibody forming cells. Immunology 14:599–608

Stockinger (1978) Negative Rückkoppelungsmechanismen des Immunsystems. Johannes Gutenberg Universität Mainz, Mainz, Germany

Zaalberg OB (1964) A simple method for detecting single antibody-forming cells. Nature 202:1231

Inhibition of Dihydro-Orotate Dehydrogenase

Baldwin J, Michnoff CH, Malmquist NA, White J, Roth MG, Rathod PK, Phillips MA (2005) High-throughput screening for potent and selective inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase. J Biol Chem 280:21847–21853

Bruneau JM, Yea CM, Spinella-Jaegle S, Fudali C, Woodward K, Robson PA, Sautès C, Westwood R, Kuo EA, Williamson RA, Ruuth E (1998) Purification of human dihydro-orotate dehydrogenase and its inhibition by A77 1726, the active metabolite of leflunomide. Biochem J 336:299–303

Diao Y, Lu W, Huangtao J, Zhu J, Han L, Xu M, Gao R, Shen X, Zhao Z, Liu X, Xu Y, Huang J, Li H (2012) Discovery of diverse human dihydroorotate dehydrogenase inhibitors as immunosuppressive agents by structure-based virtual screening. J Med Chem 55:8341–8349

Graul A, Castañer J (1998) Leflunomide. Drugs Future 23:827–837

Herrmann ML, Schleyerbach R, Kirschbaum BJ (2000) Leflunomide: an immunomodulatory drug for the treatment of rheumatoid arthritis and other autoimmune diseases. Immunopharmacology 47:273–289

Kamyingkird K, Cao S, Masatani T, Moumont PFA, Vudriko P, Mousa AAEM, Terkawi MA, Nishikawa Y, Igarashi I, Xuan X (2013) Babesia bovis dihydroorotate dehydrogenase (BboDHODH) is a novel molecular target of drug for bovine babeosis. J Vet Med Sci 76:323–330

Knecht W, Löffler M (1998) Species-related inhibition of human and rat dihydroorotate dehydrogenase by immunosuppressive isoxazol and cinchoninic acid derivatives. Biochem Pharmacol 56:1259–1264

Knecht W, Bergjohann U, Gonski S, Kirschbaum B, Löffler M (1996) Functional expression of a fragment of human dihydro-orotate dehydrogenase by means of the baculovirus vector system, and kinetic investigation of the purified enzyme. Eur J Biochem 240:292–301

Knecht W, Altekruse D, Rotgeri A, Gonski S, Löffler M (1997) Rat dihydroorotate dehydrogenase: isolation of the recombinant enzyme from mitochondria of insect cells. Protein Expr Purif 10:89–99

Liu S, Neidhardt EA, Grossman TH, Ocain T, Clardy J (2000) Structures of human dihydroorotate dehydrogenase in complex with antiproliferative agents. Struct Fold Des 8:25–33

Lukens AK, Ross LS, Heidebrecht R, Gamo FJ, Lafuente-Monasterio MJ, Booker ML, Hartl DL, Wiegand RC (2014) Wirth DF Harnessing evolutionary fitness in *Plasmodium falciparum* for drug discovery and suppressing resistance. Proc Natl Acad Sci USA 111: 799–804

Rückemann K, Fairbanks LD, Carrey EA, Hawrylowicz CM, Richards DF, Kirschbaum B, Simmonds HA (1998) Leflunomide inhibits pyrimidine de novo synthesis in mitogen-stimulated T-lymphocytes from healthy humans. J Biol Chem 273:21682–21691

Schorlemmer HU, Milbert U, Haun G, Wunschel M, Zeitter D, Schleyerbach R (1998) De novo pyrimidine biosynthesis in Jurkat T cells is inhibited by leflunomide's primary metabolite A77–1726 at the level of dihydroorotate dehydrogenase. Int J Immunother 14:193–204

General Considerations

Blaho VA, Hla T (2014) An update on the biology of sphingosine-1-phosphate receptors. J Lipid Res. doi: 10.1194/jlr.R046300

Chen XL, Grey JY, Thomas S, Qiu FH, Medford RM, Wasserman MA, Kunsch C (2004) Sphingosine kinase-1 mediates TNF-α induced MCP-1 gene expression in endothelial cells: upregulation by oscillatory flow. Am J Physiol 287:H1452–H1458

Chun J, Rosen H (2006) Lysophospholipid receptors as potential drug targets in tissue transplantation and autoimmune diseases. Curr Pharm Des 12:161–171

Cummings RJ, Parinandi NL, Zaiman A, Wang L, Usatyuk PV, Garcia JGN, Natarajan V (2002) Phospholipase D activation by sphingosine 1-phosphate regulates interleukin-8 secretion in human bronchial epithelial cells. J Biol Chem 277:30227–30235

Cyster JG (2005) Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu Rev Immunol 23:127–159

Deguchi H, Yegneswaran S, Griffin JH (2004) Sphingolipids as bioactive regulators of thrombin generation. J Biol Chem 279:12036–12042

Franzen R, Fabbro D, Aschrafi A, Pfeilschifter J, Huwiler A (2002a) Nitric oxide induces degradation of the neutral ceramidase in rat mesangial cells and is counterregulated by protein kinase C. J Biol Chem 277:46184–46190

Franzen R, Pfeilschifter J, Huwiler A (2002b) Nitric oxide induces neutral ceramidase degradation by the ubiquitin/proteasome complex in renal mesangial cell cultures. FEBS Lett 552:441–444

Gardell SE, Dubin AE, Chun J (2006) Emerging medical roles for lysophospholipid signaling. Trends Mol Med 12:65–75

Hannun YA, Obeid LM (1995) Ceramide: an intracellular signal for apoptosis. Trends Biol Sci 20:73–77

Hofmann K, Dixit VM (1998) Ceramide in apoptosis–does it really matter? Trends Biol Sci 23:374–377

Huwiler A, Pfeilschifter J, van den Bosch (1999a) Nitric oxide donors induce stress signaling via ceramide formation in rat renal mesangial cells. J Biol Chem 274:7190–7195

Huwiler A, Dorsch S, Briner V, van den Bosch H, Pfeilschifter J (1999b) Nitric oxide stimulates ceramide formation in glomerular endothelial cells. Biochem Biophys Res Commun 258:60–65

Huwiler A, Pfeilschifter J (2006) Altering the sphingosine-1-phosphate/ceramide balance: a promising approach to tumor therapy. Curr Pharm Des 12:4625–4635

Kee TH, Vit P, Melendez AJ (2005) Sphingosine kinase signaling in immune cells. Clin Exp Pharmacol Physiol 32:153–161

Lee H, Lin CI, Liao JJ, Lee YW, Yang HY, Lee CY, Hsu HY, Wu HL (2004) Lysophospholipids increase ICAM-1 expression in HUVEC through a G_i- und NF-κB-dependent mechanism. Am J Physiol 287: C1657–C1666

MacKinnon AC, Buckley A, Chilvers ER, Rossi AG, Haslett C, Sethi T (2002) Sphingosine kinase: a point of convergence in the action of diverse neutrophil priming agents. J Immunol 169:6394–6400

Mathias S, Pena LA, Kolesnick RN (1998) Signal transduction of stress via ceramide. Biochem J 335:465–480

Peng X, Hassoun PM, Sammani S, McVerry BJ, Burne MJ, Rabb H, Pearse D, Tuder RM, Garcia JGN (2004) Protective effects of sphingosine 1-phsophate in murine endotoxin-induced inflammatory lung injury. Am J Respir Crit Care Med 169:1245–1251

Prieschl EE, Csonga R, Novotny V, Kikuchi GE, Baumruker T (1999) The balance between sphingosine and sphingosine-1-phosphate is decisive for mast cell activation after Fcε receptor I triggering. J Exp Med 190:1–8

Pyne S, Pyne NJ (2000) Sphingosine 1-phosphate signaling in mammalian cells. Biochem J 349:385–402

Rosen H, Liao J (2003) Sphingosine 1-phosphate pathway therapeutics: a lipid ligand-receptor paradigm. Curr Opin Chem Biol 7:461–46

Rosen H, Stevens RC, Hanson M, Roberts E, Oldstone MB (2013) Sphingosine-1-phosphate and its receptors: structure, signaling, and influence. Annu Rev Biochem 82:637–662

Taha TA, Hannun YA, Obeid LM (2006) Sphingosine kinase: biochemical and cellular regulation and role in disease. J Biochem Mol Biol 39:113–131

Watterson KR, Ratz PH, Spiegel S (2005) The role of sphingosine-1-phosphate in smooth muscle contraction. Cell Signal 17:289–298

Binding to Sphingosine 1-Phosphate Receptors

Albert R, Hinterding K, Brinkmann V, Guerini D, Müller-Hartwieg C, Knecht H, Simeon C, Streiff M, Wagner T, Welzenbach K, Zecri F, Zollinger M, Cooke N, Francotte E (2005) The novel immunomodulator FTY720 is phosphorylated in rats and humans to form a single stereoisomer. Identification, chemical proof, and biological characterization of the biologically active species and its enantiomer. J Med Chem 48:5373–5377

Angst D, Janser P, Quancard J, Buehlmayer P, Berst F, Oberer L, Beerli C, Streiff M, Pally C, Hersperger R, Bruns C, Bassilana F, Bollbuck B (2012) An oral sphingosine 1-phosphate receptor 1 ($S1P_1$) antagonist prodrug with efficacy in vivo: discovery, synthesis, and evaluation. J Med Chem 55:9722–9734

Bandhuvula P, Tam YY, Oskoulan B, Saba JD (2005) The immune modulator FTY720 inhibits sphingosine-1-phosphate lyase activity. J Biol Chem 280:33697–33700

Bolli MH, Lescop C, Nayler O (2011) Synthetic sphingosine 1-phosphate receptor modulators – opportunities and potential pitfalls. Curr Top Med Chem 11:726–757

Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457

Brinkmann V, Billich A, Baumraker T, Heining P, Schmouder R, Rancis G, Aradhye S, Burtin P (2010) Fingolimod (FTY720): discovery and development of an oral drug to treat multiple sclerosis. Nat Rev Drug Discov 9:883–897

Brunkhorst R, Vutukuri R, Pfeilschifter W (2014) Fingolimod for the treatment of neurological diseases-state of play and future perspectives. Front Cell Neurosci 8:283 doi: 10.3389/fncel.2014.00283

Chiba K (2005) FTY720, a new class of immunomodulators, inhibits lymphocyte egress from secondary lymphoid tissues and thymus by agonistic activity at sphingosine 1-phosphate receptors. Pharmacol Ther 108:308–319

Chiba C, Kataoka H, Seki N, Maeda Y, Sugahara K (2011) Fingolimod (FTY720), the sphingosine 1-phosphate receptor modulator, as a new therapeutic drug in multiple sclerosis. Inflamm Regen 31:167–174

Clemens JJ, Davis MD, Lynch KR, Macdonald TL (2005) Synthesis of 4(5)-phenylimidazole-based analogues of sphingosine 1-phosphate and FTY720: discovery of potent $S1P_1$ receptor agonists. Bioorg Med Chem Lett 15:3568–3572

Colandrea VJ, Legiec IE, Huo P, Yan L, Hale JJ, Mills SG, Bergstrom J, Card D, Chebret G, Hajdu R, Keohane CA, Milligan JA, Rosenbach MJ, Shei GJ, Mandala SM (2006) 2,5-Disubstituted pyrrolidines carboxylates as potent, orally active sphingosine-1-phosphate (S1P) receptor agonists. Bioorg Med Chem Lett 16:2905–2908

Davis MD, Clemens JJ, Macdonald TL, Lynch KR (2005) Sphingosine 1-phosphate analogs as receptor antagonists. J Biol Chem 280:9833–9844

Forrest M, Sun SY, Hajdu R, Bergstrom J, Card D, Doherty G, Hale J, Keohane C, Meyers C, Milligan J, Mills S, Nomura H, Rosen H, Rosenbach M, Shei GJ, Singer II, Tian M, West S, White V, Xie J, Proia RL, Mandala S (2004) Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate agonists in rodents are mediated via distinct receptor subtypes. J Pharmacol Exp Ther 309:758–768

Foss FW Jr, Clemens JJ, Davis MD, Snyder AH, Zigler MA, Lynch KR, Macdonald TL (2005) Synthesis, stability, and implications of phosphothioate agonists of sphingosine-1-phosphate receptors. Bioorg Med Chem Lett 15:4470–4474

Fujita T, Inoue K, Yamamoto S, Ikumoto T, Sasaki S, Toyama R, Chiba K, Hoshima Y, Okumato T (1994) Fungal metabolites. Part II. A potent immunosuppressive activity found in *Isaria sinclairii* metabolite. J Antibiot 47:208–215

Galicia-Rosas G, Pikor N, Schwartz JA, Rojas O, Jian A, Summers-Deluca L, Ostrrowski M, Nuesslein-Hildesheim B, Gommerman JL (2012) A sphingosine-1-phosphate receptor-1 directed agonist reduces central nervous system inflammation in a plasmacytoid dendritic cell-dependent manner. J Immunol 189:3700–3706

Gräler MH, Goetzl EJ (2004) The immunosuppressant FTY720 down-regulates sphingosine 1-phosphate G-protein-coupled receptors. FASEB J 18:551–553

Guerrero M, Poddutoori R, Urbano M, Peng X, Spicer TP, Chase PS, Hodder PS, Schaeffer M-T, Brown S, Rosen H, Roberts E (2013) Discovery, design and synthesis of a selective S1P3 receptor allosteric agonist. Bioorg Med Chem 23:6346–6349

Habicht A, Clarkson MR, Yang J, Henderson J, Brinkmann V, Fernandes S, Jurewicz M, Yuan X, Sayegh MH (2005) Novel insights into the mechanism of action of FTY720 in a transgenic model of allograft rejection: implications for therapy of chronic rejection. J Immunol 176:36–42

Hale JJ, Doherty G, Toth L, Li Z, Mills SG, Hajdu R, Keohane CA, Rosenbach M, Milligan J, Shei GJ, Chrebet G, Bergstrom J, Card D, Rosen H, Mandala S (2004a) The discovery of 3-(*N*-alkyl) aminopropylphosphonic acids as potent S1P receptor agonists. Bioorg Med Chem Lett 14:3495–3499

Hale JJ, Yan L, Neway WE, Hajdu R, Bergstrom JD, Milligan JA, Shei GJ, Chrebet GL, Thornton RA, Card D, Rosenbach E, Rosen H, Mandala S (2004b) Synthesis, stereochemical determination and biochemical characterization of the enantiomeric phosphate esters of the novel immunosuppressive agent FTY720. Bioorg Med Chem 12:4803–4807

Hale JJ, Neway W, Mills SG, Hajdu R, Keohane CA, Rosenbach M, Milligan J, Shei GJ, Chrebet G, Bergstrom J, Card D, Koo GC, Koprak SL, Jackson JJ, Rosen H, Mandala S (2004c) Potent S1P receptor agonists replicate the pharmacologic actions of the novel immune modulator FTY720. Bioorg Med Chem Lett 14:3351–3355

Im DS, Heise CE, Ancellin N, O'Dowd BF, Shei GJ, Heavens RP, Rigby MR, Hla T, Mandala S, McAllister G, George SR, Lynch KR (2000) Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. J Biol Chem 275:14281–14286

Im DS, Clemens J, Macdonald T, Lynch KR (2001) Characterization of the human and mouse sphingosine 1-phosphate receptor, $S1P_5$ (Edg-8): structure-activity relationship of sphingosine 1-phosphate receptors. Biochemistry 40:14053–14060

Jin J, Hu J, Zhou W, Wang X, Xiao Q, Xue N, Yin D, Chen X (2014) Development of a selective $S1P_1$ receptor agonist, Syl930, as a potential therapeutic agent for autoimmune encephalitis. Biochem Pharmacol 90:50–61

Jo E, Sanna MG, Gonzalez-Cabrera PJ, Thangada S, Tigyl G, Osborne DA, Hla T, Parill ASL, Rosen H (2005) $S1P_1$-selective in vivo-active agonists from high-throughput screening: off-the-shelf chemical probes of receptor interactions, signaling and fate. Chem Biol 12:703–715

Kennedy PC, Zhu R, Huang T, Tomsig JL, Mathews TP, David M, Peyruchard OO, Macdonald TL, Lynch KR (2011) Characterisation of a sphingosine 1-phosphate receptor antagonist prodrug. J Pharmacol Exp Ther 338:879–889

Kimura T, Tomura H, Mogi C, Kuwabara A, Ishiwara M, Shibasawa K, Sato K, Ohwada S, Im DS, Kurose H, Ishizuka T, Murakami M, Okajima F (2006) Sphingosine 1-phosphate receptors mediate stimulatory and inhibitory signalings for expression of adhesion molecules in endothelial cells. Cell Signal 18:841–850

Kitano M, Hla T, Sekiguchi M, Kawahito Y, Yoshimura R, Miyazawa K, Iwasaki T, Sano H (2006) Sphingosine 1-phosphate/sphingosine 1-phosphate receptor 1 signaling in rheumatoid synovium. Regulation of synovial proliferation and inflammatory gene expression. Arthritis Rheum 54:742–753

Kiuchi M, Adachi K, Tomatsu A, Chino M, Takeda S, Tanaka Y, Maeda Y, Sato N, Mitsutomi N, Sugahara K, Chiba K (2005) Asymmetric synthesis and biological evaluation of the enantiomeric isomers of the immunosuppressive FTY720-phosphate. Bioorg Med Chem 13:425–432

Komiya T, Sato K, Shioya H, Inagaki Y, Hagiya H, Kozaki R, Imai M, Takada Y, Maeda T, Kurata H, Kurono M, Suzuki R, Otsuki K, Habashita H, Nakade S (2012) Efficacy and immunomodulatory actions of ONO-4641, a novel selective agonist for sphingosine 1-phosphate receptors 1 and 5, in pre-clinical models of multiple sclerosis. Clin Exp Immunol 171:454–62

Kon J, Sato K, Watanabe T, Tomura H, Kuwabara A, Kimura T, Taman KI, Ishizuka T, Murata Nkanda T, Kobayashi I, Ohta H, Ui M, Okajima F (1999) Comparison of intrinsic activities of the putative sphingosine 1-phosphate receptor subtypes to regulate several signaling pathways in their transfected Chinese hamster ovary cells. J Biol Chem 274:23940–23947

Kunzendorf U, Ziegler E, Kabelitz D (2004) FTY720-the first compound of a new promising class of immunosuppressive drugs. Nephrol Dial Transplant 19:1677–1681

Lepley D, Paik JH, Hla T, Ferrer F (2005) The G protein-coupled receptor $S1P_2$ regulates Rho/Rho kinase pathway to inhibit tumor cell migration. Cancer Res 65:3788–3795

Li Z, Chen W, Hale JJ, Lynch CL, Mills SG, Hajdu R, Keohans CA, Rosenbach MJ, Milligan JA, Shei GJ, Chrebet G, Parent SA, Bergstrom J, Card D, Forrest M, Quackenbush EJ, Wickham LA, Vargas H, Evans TRM, Rosen H, Mandala S (2005) Discovery of potent 3,5-diphenyl-1,2,4-oxadiazole sphingosine 1-phosphate ($S1P_1$) receptor agonists with exceptional selectivity against $S1P_2$ and $S1P_3$. J Med Chem 48:6169–6173

Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thronton R, Shei GJ, Card D, Keohane CA, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2002) Alteration of lymphocyte trafficking by sphingosine receptor agonists. Science 296:346–349

Meyer zu Heringdorf D, Lass H, Alemany R, Laser KT, Neumann E, Zhang C, Schmidt M, Rauen U, Jakobs KH, van Koppen CJ (1998) Sphingosine kinase-mediated Ca2+ signalling by G-protein-coupled receptors. EMBO J 17:2830–2837

Murata N, Sato K, Kon J, Tomura H, Okajima F (2000) Quantitative measurement of sphingosine 1-phosphate by radioreceptor-binding assay. Anal Biochem 282:115–120

Ren F, Deng G, Wang H, Luan L, Meng Q, Xu Q, Xu H, Xu X, Zhang H, Zhao B, Li C, Guo TB, Yang J, Zhang W, Zhao Y, Jia Q, Lu H, Xiang J-N, Elliott JD, Lin X (2012) Discovery of novel 1,2,4-thiadiazole derivatives as potent, orally active agonists of sphingosine 1-phosphate receptor subtype 1 ($S1P_1$). J Med Chem 55:4286–4296

Roberts E, Guerrero M, Urbano M, Rosen H (2013) Sphingosine 1-phosphate receptor agonists: a patent review (2010–2012). Expert Opin Ther Pat 23:817–841

Sanada Y, Mizushima T, Kai Y, Nishimura J, Hagiya H, Kurata H, Mizuno H, Uejima E, Ito T (2011) Therapeutic effects of novel sphingosine-1-phosphate receptor agonist W-061 in murine DSS colitis. PLoS One 6: e23933

Sanna MG, Liao J, Jo E, Alfonso C, Ahn MY, Peterson MS, Webb B, Lefebvre S, Chun J, Gray N, Rosen H (2004) Sphingosine 1-phosphate (S1P) receptor subtypes $S1P_1$ and $S1P_3$, respectively, regulate lymphocyte recirculation and heart rate. J Biol Chem 279:13839–13848

Satsu H, Schaeffer M-T, Guerro M, Saldana A, Eberhardt C, Hodder P, Cayanan S, Bhhatarai B, Roberts E, Rosen H, Brown SJ (2013) A sphingosine 1-phosphate receptor 2 selective allosteric agonist. Bioorg Med Chem 21:5373–5382

Sawicka E, Zuany-Amorim C, Manlius C, Trifilieff A, Brinkmann V, Kemeny DM, Walker C (2003) Inhibition of Th1- and Th2-mediated airway inflammation by the sphingosine 1-phosphate receptor agonist FTY720. J Immunol 171:6206–6214

Sawicka E, Dubois G, Jarai G, Edwards M, Thomas M, Nicholls A, Albert R, Newson C, Brinkmann V, Walker C (2005) The sphingosine 1-phosphate receptor agonist FTY720 differentially affects the sequestration of $CD4^+/CD25^+$ T-regulatory cells and enhances their functional activity. J Immunol 175:7973–7980

Sobel K, Menyhart K, Killer N, Renault B, Bauer Y, Studer R, Steiner B, Bolli MH, Nayler O, Gatfield J (2013) Sphingosine 1-phosphate (S1P) receptor agonists mediate pro-fibrotic responses in normal human lung fibroblasts via $S1P_2$ and $S1P_3$ receptors and Smad-independent signaling. J Biol Chem 288:14839–14851

Takasugi N, Sasaki T, Ebinuma I, Osawa S, Isshiki H, Takeo K, Tomita T, Iwatsubo T (2013) FTY720/fingolimod, a sphingosine analogue, reduces amyloid-β production in neurons. PLoS One 8:e64050

Xin C, Ren S, Pfeilschifter J, Huwiler A (2004) Heterologous desensitization of the sphingosine 1-phosphate receptors by purinoceptor activation in renal mesangial cells. Br J Pharmacol 143:581–589

Xin C, Ren S, Eberhardt W, Pfeilschifter J, Huwiler A (2006) The immunomodulators FTY720 and its phosphorylated derivative activate the Smad signaling cascade and upregulate connective tissue growth factor and collagen IV expression in renal mesangial cells. Br J Pharmacol 147:164–174

Yamamoto R, Okada Y, Hirose J, Koshika T, Kawato Y, Maeda M, Saito R, Hattori K, Harada H, Nagasaka Y, Morokata T (2014) ASP4058, a novel agonist for sphingosine 1-phosphate receptors 1 and 5, ameliorates rodent experimental autoimmune encephalomyelitis with a favorable safety profile. PLoS One 8:e110819

Zhang L, Wang HD, Ji XJ, Cong ZX, Zhu JH, Zhou Y (2013) FTY720 for cancer therapy (review). Oncol Rep 30:2571–2578

Zhou H, Murthy K (2004) Distinctive G protein-dependent signaling in smooth muscle by sphingosine 1-phosphate receptors $S1P_1$ and $S1P_2$. Am J Physiol 286:C1130–C1138

Zhou C, Ling MT, Lee TKW, Man K, Wang X, Wong YC (2006) FTY720, a fungus metabolite, inhibits invasion ability of androgen-independent prostate cancer cells through inactivation of RhoA-GTPase. Cancer Lett 223:36–47

Zhu J, Liu Y, Pi Y, Jia L, Wang L, Huang Y (2014) Systemic application of sphingosine 1-phosphate receptor 1 immunomodulator inhibits corneal allograft rejection in mice. Acta Opthalmol 92:e12–21

Sphingosine Kinase Activation Assay

Billich A, Bornancin F, Mechtcheriakova D, Natt F, Huesken D, Baumruker T (2005) Basal and induced sphingosine kinase 1 activity in A549 carcinoma cells: function in cell survival and IL-1 β and TNF-α induced production of inflammatory mediators. Cell Signal 17:1203–1217

Ceccom J, Loukh N, Lauwers-Cances V, Touriol C, Nicaise Y, Gentil C, Uro-Coste E, Pitson S, Maurage CA, Duyckaerts C, Cuvillier O, Delisle M-B (2014) Reduced sphingosine kinase-1 and enhanced sphingosine 1-phosphate lyase expression demonstrate deregulated sphingosine 1-phosphate signaling in Alzheimer's disease. Acta Neuropathol Commun 2:1–10

De Palma C, Meacci E, Perrotta C, Bruni P, Clementi E (2006) Endothelial nitric oxide synthase activation by tumor necrosis factor a through neutral sphingomyelinase 2, sphingosine kinase 1, and sphingosine 1 receptors. Arterioscler Thromb Vasc Biol 26:99–105

Döll F, Pfeilschifter J, Huwiler A (2005) The epidermal growth factor stimulates sphingokinase-1 expression and activity in the human mammary carcinoma cell line MCF7. Biochim Biophys Acta 1738:72–81

Gao P, Smith CD (2011) Ablation of sphingosine kinase-2 inhibits tumor cell proliferation and migration. Mol Cancer Res 9:1509–1519

Hait NC, Sarkar S, Le Stunff H, Mikami A, Maceyka M, Milstien S, Spiegel S (2005) Role of sphingosine kinase 2 in cell migration toward epidermal growth factor. J Biol Chem 280:29462–29469

Huwiler A, Döll F, Ren S, Klawitter S, Greening A, Römer I, Bubnova S, Reinsberg L, Pfeilschifter J (2006) Histamine increases sphingosine kinase-1 expression and activity in the human endothelial cell line E.A.hy926 by a PCK-α-dependent mechanism. Biochim Biophys Acta 1761:367–376

Igarashi N, Okada T, Hayashi S, Fujita T, Jahangeer S, Nakamura SI (2003) Sphingosine kinase 2 is a nuclear protein and inhibits DNA synthesis. J Biol Chem 278:46832–46839

Kharel Y, Lee S, Snyder AH, Sheasley-O'Neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KSK, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR (2005) Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. J Biol Chem 280:36856–36872

Kohama T, Olivera A, Edsall L, Nagiec MM, Dickson R, Spiegel S (1998) Molecular cloning and functional characterization of murine sphingosine kinase. J Biol Chem 273:23722–23728

Kunkel GT, Maceyka M, Milstien S, Spiegel S (2013) Targeting the sphingosine-1-phosphate axis in cancer, inflammation and beyond. Nat Rev Drug Discov 12:688–702

Liu H, Sugiura M, Nava VE, Edsall LC, Kono K, Poultoni S, Milstien S, Kohama T, Spiegel S (2000) Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform. J Biol Chem 275:19513–19520

Liu H, Toman RE, Goparaju SK, Maceyka M, Nava VE, Sankala H, Payne SG, Bektas M, Ishii I, Chun J, Milstien S, Spiegel S (2003) Sphingosine kinase 2 is a putative BH3-only protein that induces apoptosis. J Biol Chem 278:40330–40336

Nava VE, Lacana E, Poulton S, Liu H, Sugiura M, Kono K, Milstien S, Kohama T, Spiegel S (2000) Functional characterization of human sphingosine kinase-1. FEBS Lett 473:81–84

Neubauer HA, Pitson SM (2013) Roles, regulation and inhibitors of sphingosine kinase 2. FEBS J 280:5317–5336

Okada T, Ding G, Sonoda H, Kajimoto T, Haga Y, Khosrowbeygi A, Goa S, Miwa N, Jahangeer S, Nakamura SI (2005) Involvement of N-terminal-extended form of sphingosine kinase 2 in serum-dependent regulation of cell proliferation and apoptosis. J Biol Chem 280:36318–36325

Olivera A, Barlow KD, Spiegel S (2000) Assaying sphingosinekinase activity. Methods Enzymol 311:215–223

Paugh SW, Payne SG, Barbour SE, Milstien S, Spiegel S (2003) The immunosuppressant FTY720 is phosphorylated by sphingosine kinase type 2. FEBS Lett 554:189–193

Plano D, Amin S, Sharma AK (2014) Importance of sphingosine kinase (SphK) as a target in developing cancer

therapeutics and recent developments in the synthesis of novel SphK inhibitors. J Med Chem 57:5509–5524
Sanchez T, Estrada-Hernandez T, Paik JH, Wu MT, Venkataraman K, Brinkmann V, Claffey K, Hla T (2003) Phosphorylation and action of the immunomodulator FTY720 inhibits vascular endothelial cell growth factor-induced vascular permeability. J Biol Chem 278:27281–27290
Shen H, Giordano F, Wu Y, Chan J, Zhu C, Liosevic I, Wu X, Yao K, Chen B, Baumgart T, Sieburth D, de Camilli P (2014) Coupling between endocytosis and sphingosine kinase 1 recruitment. Nat Cell Biol 16:652–662
Tamashiro PM, Furuya H, Shimizu Y, Kawamori T (2014) Sphingosine kinase 1 mediates head and neck squamous cell carcinoma invasion through sphingosine 1-phosphate receptor 1. Cancer Cell Int 14:76
Tonellli F, Alossaimi M, Natarajan V, Gorshikova I, Berdyshev E, Bittman R, Watson DG, Pyne S, Pyne NJ (2013) The roles of sphingosine kinase 1 and 2 in regulating the metabolome and survival of prostate cancer cells. Biomolecules 3:316–333
Tous M, Ferrer-Lorente R, Badimon L (2014) Selective inhibition of sphingosine kinase-1 protects adipose tissue against LPS-induced inflammatory response in Zucker diabetic fatty rats. Am J Physiol Endocrinol Metab 307:E437–446
Zemann B, Kinzel B, Müller M, Reuschel R, Mechtcheriakowa D, Urtz N, Bomancin F, Baumruker T, Billich A (2006) Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood 107:1454–1458
Zhang L, Urtz N, Gaertner F, Legate KR, Petzold T, Lorenz M, Mazharian A, Watson SP, Massberg S (2013) Sphingosine kinase 2 (Sphk2) regulates platelet biogenesis by providing intracellular sphingosine 1-phosphate (S1P). Blood 122:791–802

Lymphocyte Trafficking After Sphingosine 1-Phosphate Receptor Agonists

Brinkmann V, Davis MD, Heise CE, Albert R, Cottens S, Hof R, Bruns C, Prieschl E, Baumruker T, Hiestand P, Foster CA, Zollinger M, Lynch KR (2002) The immune modulator FTY720 targets sphingosine 1-phosphate receptors. J Biol Chem 277:21453–21457
Chiba K, Yanagawa Y, Masubuchi Y, Karaoka H, Kawaguchi T, Ohtsuki M, Hoshino Y (1998) FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. I. FTY720 selectively decreases the number of circulating mature lymphocytes by acceleration of lymphocyte homing. J Immunol 160:5037–5044
Chiba K, Maeda Y, Seki N, Kataoka H, Sugahara K (2014) Role of sphingosine 1-phosphate (S1P) and effects of fingolimod, an S1P receptor 1 antagonist in lymphatic circulation and autoimmune disease. AIMS Mol Sci 1:162–182
Forrest M, Sun SY, Hajdu R, Bergstrom J, Card D, Doherty G, Hale J, Keohane C, Meyers C, Milligan J, Mills S, Nomura H, Rosen H, Rosenbach M, Shei GJ, Singer II, Tian M, West S, White V, Xie J, Proia RL, Mandala S (2004) Immune cell regulation and cardiovascular effects of sphingosine 1-phosphate agonists in rodents are mediated via distinct receptor subtypes. J Pharmacol Exp Ther 309:758–768
Fueller M, Wang DA, Tigyi G, Siess W (2003) Activation of human monocytic cells by lysophosphatidic acid and sphingosine-1-phosphate. Cell Signal 15:367–375
Hait NC, Sarkar S, Le Stunff H, Mikami A, Maceyka M, Milstien S, Spiegel S (2005) Role of sphingosine kinase 2 in cell migration toward epidermal growth factor. J Biol Chem 280:29462–29469
Henning G, Ohl L, Junt T, Reiterer P, Brinkmann V, Nakano H, Hohenberger W, Lipp M, Förster R (2001) CC chemokine receptor 7-dependent and -independent pathways for lymphocyte homing: modulation by FTY720. J Exp Med 194:1875–1881
Huwiler A, Döll F, Ren S, Klawitter S, Greening A, Römer I, Bubnova S, Reinsberg L, Pfeilschifter J (2006) Histamine increases sphingosine kinase-1 expression and activity in the human endothelial cell line E.A.hy926 by a PCK-α-dependent mechanism. Biochim Biophys Acta 1761:367–376
Kawa S, Kimura S, Hakomori SI, Igarashi Y (1997) Inhibition of chemotactic motility and trans-endothelial migration of human neutrophils by sphingosine 1-phosphate. FEBS Lett 420:196–200
Kharel Y, Lee S, Snyder AH, Sheasley-O'Neill SL, Morris MA, Setiady Y, Zhu R, Zigler MA, Burcin TL, Ley K, Tung KSK, Engelhard VH, Macdonald TL, Pearson-White S, Lynch KR (2005) Sphingosine kinase 2 is required for modulation of lymphocyte traffic by FTY720. J Biol Chem 280:36856–36872
Kimura T, Boehmler AM, Seitz G, Kuçi S, Wiesner T, Brinkmann V, Kanz L, Möhle R (2004) The sphingosine 1-phosphate receptor agonist FTY720 supports CXCR4-dependent migration and bone marrow homing of human $CD34^+$ progenitor cells. Blood 103:4478–4486
Kunisawa J, Kurashima Y, Gohda M, Higuchi M, Ishikawa I, Miura F, Ogahara I, Kiyono H (2007) Sphingosine 1-phosphate regulates peritoneal B-cell trafficking for subsequent intestinal IgA production. Blood 109:3749–3756
Mandala S, Hajdu R, Bergstrom J, Quackenbush E, Xie J, Milligan J, Thronton R, Shei GJ, Card D, Keohane CA, Rosenbach M, Hale J, Lynch CL, Rupprecht K, Parsons W, Rosen H (2000) Alteration of lymphocyte trafficking by sphingosine receptor agonists. Science 296:346–349
Matloubian M, Lo CG, Cinamom G, Lesneski MJ, Xu Y, Brinkmann V, Allende ML, Proia RL, Cyster JG (2004)

Lymphocyte egress from thymus and peripheral lymphoid organs is dependent on S1P receptor 1. Nature 427:355–360

Roviezzo F, del Galdo F, Abbate G, Bucci M, D'Agostino B, Antunes E, de Dominicis G, Parente L, Rossi F, Cirino G, de Palma R (2004) Human eosinophil chemotaxis and selective in vivo recruitment by sphingosine 1-phosphate. Proc Natl Acad Sci U S A 101:11170–11175

Thangada S, Khanna KM, Blaho VA, Oo ML, Im D-S, Guo C, Lefrancois L, Hla T (2010) Cell-surface residence of sphingosine 1-phosphate receptor 1 on lymphocytes determines the lymphocyte egress kinetics. J Exp Med 207:1475–1483

Yanagawa Y, Sugahara K, Kataoka H, Kawaguchi T, Masubuchi Y, Chiba K (1998a) FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. II. FTY720 prolongs allograft survival by decreasing T cell infiltration into grafts but not cytokine production in vivo. J Immunol 160:5493–5499

Yanagawa Y, Masubuchi Y, Chiba K (1998b) FTY720, a novel immunosuppressant, induces sequestration of circulating mature lymphocytes by acceleration of lymphocyte homing in rats. Immunology 95:591–594

Yang D, Sun Y-Y, Bhaumik SK, Li Y, Baumann JM, Lin X, Zhang Y, Lin S-H, Dunn RS, Liu C-Y, Shie F-S, Lee Y-H, Wills-Karp M, Chougnet CA, Kallapur SG, Lewkowich AP, Lindquist DM, Murali-Krishna K, Kuan C-Y (2014) Blocking lymphocyte trafficking with FTY720 prevents inflammation-sensitized hypoxic-ischemic brain injury in newborns. J Neurosci 34:16467–15481

Zemann B, Kinzel B, Müller M, Reuschel R, Mechtcheriakowa D, Urtz N, Bomancin F, Baumruker T, Billich A (2006) Sphingosine kinase type 2 is essential for lymphopenia induced by the immunomodulatory drug FTY720. Blood 107:1454–1458

Spontaneous Autoimmune Diseases in Animals

Adipue IA, Wilcox JT, King C, Rice CAY, Shaum KM, Suard CM, ten Brink E, Miller SD, McMahon EJ (2011) Characterisation of a novel and spontaneous mouse model of inflammatory arthritis. Arthritis Res Ther 13:R1114

Allen EM, Thupari JN (1995) Thyroglobulin-reactive T lymphocytes in thyroiditis-prone BB/Wor rats. J Endocrinol Invest 18:45–49

Barthold DR, Kysela S, Steinberg AD (1974) Decline in suppressor T cell function with age in female NZB mice. J Immunol 112:9

Bielschowski M, Helyer BJ, Howie JB (1959) Spontaneous anemia in mice of the NZB/BL strain. Proc Univ Otago Med Sch 37:9–11

Blanchard D, Bach MA (1980) Thymic function in NZB mice. Clin Exp Immunol 42:1–9

Brezinscheck HP, Gruschwitz M, Sgone R, Moormann S, Herold M, Gershwin ME, Wick G (1993) Effects of cytokine application on glucocorticoid secretion in an animal model for systemic scleroderma. J Autoimmun 6:719–733

Cihak J, Hoffmann-Fezer G, Koller A, Kaspers B, Merkle H, Hala K, Wick G, Losch U (1995) Preferential TCR Vβ1 gene usage by autoreactive T cells in spontaneous autoimmune thyroiditis of the obese strain of chickens. J Autoimmun 8:507–520

Cole RK (1966) Hereditary hypothyroidism in domestic fowl. Genetics 13:1021–1033

Cole RK, Kite JH, Witebsky E (1968) Hereditary autoimmune thyroiditis in the fowl. Science 160:1357–1358

Cole RK, Kite JH, Wick G, Witebsky E (1970) Inherited autoimmune thyroiditis in the fowl. Poult Sci 49:480–488

Del Prete GF, Tiri A, Parronchi P, Pinchera A, Romagnani S, Ricci M, Mariotti S (1989) Thyroiditis as a model of organ specific autoimmune disease. Clin Exp Rheumatol 7(Suppl 3):S41–S46

Dietrich HM, Oliveira Dos Santos AJ, Wick G (1997) Development of spontaneous autoimmune thyroiditis in Obese strain (OS) chickens. Vet Immunol Immunopathol 57:141–146

Field JB (ed) (1983) The juvenile diabetes foundation workshop on the spontaneously diabetic BB rat: its potential for insight into human juvenile diabetes. Metabolism 32(Suppl 1):1–166

Gershwin ME, Abplanalp JJ, Castles RM, Ikeda J, van de Water J, Eklund J, Haynes D (1981) Characterization of a spontaneous disease of white leghorn chickens resembling progressive systemic sclerosis (scleroderma). J Exp Med 153:1640–1659

Giarratana N, Penna G, Adorini L (2007) Animal models of spontaneous autoimmune disease: type 1 diabetes in the non-obese diabetic mouse. Methods Mol Biol 380:285–311

Green MC, Shultz LD (1975) Motheaten, an immunodeficient mutant of the mouse. I. Genetics and pathology. J Hered 66:250–258

Hala K, Malin G, Dietrich H, Loesch U, Boeck G, Wolf H, Kaspers B, Geryk J, Falk M, Boyd RL (1996) Analysis of the initiation period of spontaneous autoimmune thyroiditis (SAT) in the obese strain (OS) of chickens. J Autoimmun 9:129–138

Hayashi Y, Kurashima C, Utsuyama M, Hirokawa K (1988) Spontaneous development of autoimmune sialadenitis in aging BDF1 mice. Am J Pathol 132:173–179

Helyer BW, Howie JB (1963) Renal disease associated with positive lupus erythematosus test in a cross-bred strain of mice. Nature 197:197

Hertler CK, Trune DR (1990) Otic capsule bony lesions in the Palmerston North autoimmune mouse. Otolaryngol Head Neck Surg 103:713–718

Holmdahl R, Jansson L, Andersson M, Jonsson R (1992) Genetic, hormonal and behavioural influence on

spontaneously developing arthritis in normal mice. Clin Exp Immunol 88:467–472
Howie JB, Helyer BJ (1968) The immunology and pathology of NZB mice. Adv Immunol 9:215–266
Itoh N, Imagawa M, Hanafusa T, Waguri M, Yamamoto K, Iwahshi A, Morikawi M, Nakajima H, Miyagawa J, Namba M, Makino S, Nagata S, Kono N, Matsuzawa Y (1997) Requirement of Fas for the development of autoimmune diabetes in nonobese diabetic mice. J Exp Med 186:613–618
Kessler HS (1968) A laboratory model for Sjögren's syndrome. Am J Pathol 52:671–685
Kroemer G, Neu N, Kuehr T, Dietrich F, Fassler R, Hala K, Wick G (1989) Immunogenetic analysis of spontaneous autoimmune thyroiditis of obese strain of chickens. Clin Immunol Immunopathol 52:202–213
Leiter EH, Prochazka M, Coleman DL (1987) Animal model of human disease. The non-obese diabetic (NOD) mouse. Am J Pathol 128:380–383
Like AA, Butler L, Williams RM, Appel MC, Weringer EJ, Rossini AA (1982) Spontaneous autoimmune diabetes mellitus in the BB rat. Diabetes 31(Suppl):7–13
Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y (1980) Breeding of a non-obese, diabetic strain of mice. Exp Anim 29:1–13
Many MC, Maniratunga S, Denef JF (1996) The non-obese diabetic (NOD) mouse: an animal model for autoimmune thyroiditis. Exp Clin Endocrinol Diabetes 104 (Suppl 3):17–20
Miyazaki A, Hanafusa T, Yamada K, Miyagawa J, Fujino-Kurihara H, Nagajima H, Nonaka K, Tarui S (1985) Predominance of T lymphocytes in pancreatic islets and spleen of pre-diabetic non-obese diabetic (NOD) mice: a longitudinal study. Clin Exp Immunol 60:622–630
Neu N, Hala K, Dietrich H, Wick G (1986) Genetic background of spontaneous autoimmune thyroiditis in the obese strain of chickens studied in hybrids with an inbred line. Int Arch Allergy Appl Immunol 80:168–173
Qi N, Liu P, Zhang Y, Wu H, Chen Y, Han D (2013) Development of a spontaneous liver disease resembling autoimmune hepatitis in mice lacking tyro3, axl and mer receptor tyrosine kinases. PLoS One 8:e66604
Quartey-Papafio R, Lund T, Chandler P, Picard J, Ozegbe P, Hutchings PR, O'Reilly L, Kioussis D, Simpson E, Cooke A (1995) Aspartate at position 57 of nonobese diabetic I-A (g7) β-chain diminishes the spontaneous incidence of insulin-dependent diabetes mellitus. J Immunol 154:5567–5575
Robison R, Tung KSK, Meeker ND, Monson FG, Teuscher C (1994) A murine model of spontaneous aspermatogenesis: linkage to H_2. J Reprod Immunol 26:251–260
Schumm-Draeger PM, Fortmeyer HP (1996) Autoimmune thyroiditis – spontaneous disease models – cat. Exp Clin Endocrinol Diabetes 104(Suppl 3):12–13
Schuurs AHWM, Verheul HAM, Wick G (1989) Spontaneous autoimmune models. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 449–485
Shultz LD (1988) Pleiotropic effects of deleterious alleles in the "motheaten" locus. Curr Top Microbiol Immunol 137:216–222
Shultz LD, Coman DR, Bailey CL, Beamer WG, Sidman CL (1984) "Viable motheaten," a new allele in the motheaten locus. Am J Pathol 116:179–192
Solleveld HA, Coolen J, Haajiman JJ (1985) Animal model of human disease: autoimmune thyroiditis. Spontaneous autoimmune thyroiditis in praomys (mastomys) coucha. Am J Pathol 119:345–349
Sone M, Nariuchi H, Saito K, Yanagita M (1995) A substrain of NZB mouse as an animal model of autoimmune inner ear disease. Hear Res 83:26–26
Su X, Zhou T, Yang P, Edwards CK III, Mountz JD (1998) Reduction of arthritis and pneumonitis in motheaten mice by soluble tumor necrosis factor receptor. Arthritis Rheum 41:139–149
Traynor SJ, Cohen JI, Morton JI, Trune DR (1992) Immunohistochemical analysis of otic capsule osteogenesis in the Palmerston North autoimmune mouse. Otolaryngol Head Neck Surg 106:196–201
Tsubata R, Tsubata T, Hiai H, Shinkura R, Matsumura R, Sumida T, Miyawaki S, Ishida H, Kumagai S, Nakao I, Honjo T (1996) Autoimmune disease of exocrine organs in immunodeficient alymphoplasia mice: a spontaneous model for Sjøgren's syndrome. Eur J Immunol 26:2742–2748
Van de Water J, Gershwin ME, Aplanalp H, Wick G, van der Mark K (1984) Serial observations and definition of mononuclear cell infiltrates in avian scleroderma, an inherited fibrotic disease of chickens. Arthritis Rheum 27:807–815
van Tienhoven A, Cole RK (1962) Endocrine disturbance in obese chickens. Anat Rev 142:111–122
Wick G, Sundick RS, Albini B (1974) The obese strain (OS) of chickens: an animal model with spontaneous autoimmune thyroiditis. Clin Immunol Immunopathol 3:272–300
Yale JF, Marliss EB (1984) Altered immunity and diabetes in the BB rat. Clin Exp Immunol 57:1–11
Yang M, Rainone A, Shi XQ, Fournier S, Zhang J (2014) A new model of spontaneous autoimmune peripheral polyneuropathy: implications for Guillain-Barre syndrome. Acta Neuropath Commun 2:5. doi: 10.1186/2051-5960-2-5

Acute Systemic Anaphylaxis in Rats

Austen KF, Brocklehurst WE (1961) Anaphylaxis in chopped guinea pig lung. J Exp Med 113:521–537
Bhattacharya BK, Delaunois AL (1955) An improved method for the perfusion of isolated lung of guinea pig. Arch Int Pharmacodyn Ther 101:495–510
Davies GE, Evans DP (1973) Studies with two new phosphodiesterase inhibitors (ICI 58,301 and ICI 63,197) on

anaphylaxis in guinea pigs, mice and rats. Int Arch Allergy 45:467–478
Elwood W, Lötvall JO, Barnes PJ, Chung KF (1992) Effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell responses in sensitized Brown-Norway rats. Am Rev Respir Dis 145:1289–1294
Herxheimer H (1952) Repeatable 'microshocks' of constant strength in guinea pig anaphylaxis. J Physiol 117:251–255
Omote M, Sakai K, Mizusawa H (1994) Acute effects of deflazacort and its metabolite 21-desacetyl-deflazacort on allergic reactions. Arzneim Forsch/Drug Res 44:149–153
Ufkes JGR, Ottenhof M (1984) Characterization of various anti-allergic agents using a new method for inducing systemic anaphylaxis in the rat. J Pharmacol Methods 11:219–226

Anti-Anaphylactic Activity (Schultz–Dale Reaction)

Anderson P, Brattsand R (1982) Protective effects of the glucocorticoid, budesonide, on lung anaphylaxis in actively sensitized guinea pigs: inhibition of the IgE – but not of the IgG – mediated anaphylaxis. Br J Pharmacol 76:139–147
Austen KF, Brocklehurst WE (1961) Anaphylaxis in chopped guinea pig lung. J Exp Med 113:521–537
Choi IS, Cui Y, Koh YA, Cho YB, Won YH (2008) Effects of dehydroepiandrosterone on the Schultz-Dale reaction and the Th2 immune response in sensitized BALB/c mice. Korean J Asthma Allergy Clin Immunol 28:121–127
Dale HH (1913) The anaphylactic reaction of plain muscle in the guinea-pig. J Pharmacol Exp Ther 4:167–223
Guhathakurta S, Gulati K, Rai N, Banerji BD, Jamil SS, Ray A (2013) An experimental study to evaluate the anti-inflammatory and immunomodulatory effects of UNIM-352, a polyherbal preparation for bronchial asthma. Med Plant Res 3:3–12
Koppel GA, Haisch KD, Spaethe SM, Schmidtke JR, Fleisch JH (1981) Schultz–Dale reaction in mouse trachea. J Pharmacol Methods 6:39–43
Laekeman GM, Herman AG, van Nueten JM (1977) Influence of different drugs on the slow response of the intestine during the Schultz–Dale reaction. Arch Int Pharmacodyn Ther 230:335
Naik SR, Bhagatb S, Shaha PD, Tarea AA, Ingawalea D, Wadekara RR (2013) Evaluation of anti-allergic and anti-anaphylactic activity of ethanolic extract of *Zizyphus jujuba* fruits in rodent. Rev Bras Farm 23:811–818
Omote M, Sakai K, Mizusawa H (1994) Acute effects of deflazacort and its metabolite 21-desacetyl-deflazacort on allergic reactions. Arzneim Forsch/Drug Res 44:149–153
Schultz WH (1910) Physiological studies in anaphylaxis. 1. The reaction of smooth muscle of the guinea-pig sensitized with horse serum. J Pharmacol Exp Ther 1:549–567

Passive Cutaneous Anaphylaxis

Babakin AA, Andrievsky G, DuBuske LM (2008) Inhibition of systemic and passive cutaneous anaphylaxis by water-soluble Fullerene 60. J Allergy Clin Immunol 123:S118
Goose J, Blair AMJN (1969) Passive cutaneous anaphylaxis in the rat, induced with two homologous reagin-like antibodies and its specific inhibition with disodium cromoglycate. Immunology 16:749–760
Griesbacher T, Lembeck F (1987) Actions of bradykinin antagonists on bradykinin-induced plasma extravasation, venoconstriction, prostaglandin E_2 release, nociceptor stimulation and contraction of the iris sphincter muscle of the rabbit. Br J Pharmacol 92:333–340
Han S-Y, Bae J-Y, Park S-H, Kim Y-H, Park JHY, Kang Y-H (2013) Resveratrol inhibits IgE-mediated basophilic mast cell degranulation and passive cutaneous anaphylaxis in mice. J Nutr. doi: 10.3945/jn.112.173302
Hitomi K, Tahara-Hanaoka S, Someya S, Fujiki A, Tada H, Sugiyama T, Shibayama S, Shibuya K, Shibuya A (2010) An immunoglobulin-like receptor, allergin-1, inhibits immunoglobulin E-mediated immediate hypersensitivity reactions. Nat Immunol 11:601–607
Katayama S, Shionoya H, Ohtake S (1975) A new simple method for extraction of extravasated dye in the skin. Jpn J Pharmacol Suppl 25:103P
Lembeck F, Griesbacher T, Eckhardt M, Henke S, Breipohl G, Knolle J (1991) New, long-acting, potent bradykinin antagonists. Br J Pharmacol 102:297–304
Miles AA, Miles EM (1952) Vascular reactions to histamine, histamine-liberator and leukotaxine in the skin of guinea pigs. J Physiol 118:228–257
Patterson R, Talbot CH, Brandfonbrener M (1971) The use of IgE mediated responses as a pharmacologic test system. The effect of disodium cromoglycate in respiratory and cutaneous reactions and in the electrocardiograms of rhesus monkeys. Int Arch Allergy Immunol 41:592–603
Saria A, Lundberg JM, Skofitsch G, Lembeck F (1983) Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, histamine and by antigen challenge. Naunyn Schmiedeberg's Arch Pharmacol 324:212–218
Watanabe N, Ovary Z (1977) Antigen and antibody detection by in vivo methods: a reevaluation of passive cutaneous anaphylactic reactions. J Immunol Methods 14:381–390
Zhu Y, Peng C, Xu JG, Liu YX, Zhu QG, Liu JY, Li FQ, Wu JH, Hu JH (2009) Participation of proteinase-activated receptor-2 in passive cutaneous anaphylaxis-induced scratching behaviour and the inhibitory effect of tacrolimus. Biol Pharm Bull 32:1173–1176

Arthus Type Immediate Hypersensitivity

Bartlett RR, Gebert U, Kerékjártó B, Schleyerbach R, Thorwart W, Weitmann KU (1989) Substituted 3-phenyl-7H-thiazolo (3,2-b)(1,2,4) triazin-7-ones as antiinflammatory agents with immunomodulating properties. Drugs Exp Clin Res 15:521–526

Horvat J, Vidic B, Kosec D, Stojic Z, Jankovic BD (1990) Suppression of Arthus and delayed hypersensitivity reactions to bovine serum albumin by dopaminergic antagonists. Period Biol 92:81–82

Kamei C, Izushi K, Adachi Y, Shimazawa M, Tasaka K (1991) Inhibitory effect of epinastine on the type II–IV allergic reactions in mice, rats and guinea pigs. Arzneim Forsch/Drug Res 41:1150–1153

Nagakawa Y, Ogawa T, Kobayashi M, Wagatsuma K, Munakata H, Umezu K, Sato S, Shibata Y, Inoue K, Ishida N (1990) Immunopharmacological studies of 4-acetylaminophenyl-acetic acid. (MS-932). Int J Immunother 6:131–140

Omote M, Sakai K, Mizusawa H (1994) Acute effects of deflazacort and its metabolite 21-desacetyl-deflazacort on allergic reactions. Arzneim Forsch/Drug Res 44:149–153

Delayed Type Hypersensitivity (DTH)

Atkinson SM, Usher PA, Kvist PH, Markholst H, Haase C, Nansen A (2012) Establishment and characterization of a sustained delayed-type hypersensitivity model with arthritic manifestations in C57/BL6J mice. Arthritis Res Ther 14:R134

Borel JF (1989) Pharmacology of cyclosporine (Sandimmune). IV. Pharmacological properties in vivo. Pharmacol Rev 41:259–371

Borel JF, Feurer C, Magnée C, Stähelin H (1977) Effects of the new anti-lymphocytic peptide cyclosporin A in animals. Immunology 32:1017–1025

Escandell JM, Recio M-C, Giner RM, Manez S, Cerda-Nicolas M, Merfort I, Rios J-L (2010) Inhibition of delayed-type sensitivity by cucurbitacin R through the curbing of lymphocyte proliferation and cytokine expression by means of nuclear factor AT translocation to the nucleus. J Pharmacol Exp Ther 232:352–363

Heriazon A, Yager JA, Sears W (2009) Mallard BA (2009) Induction of delayed-type hypersensitivity and interferon-gamma to Candida albicans and anti-hen-egg white lysozyme antibody as phenotypic markers of enhance bovine immune response. Vet Immunol Immunopathol 129:93–100

Herrmann P, Schreier MH, Borel JF, Feurer C (1988) Mast cell degranulation as a major event in the effector phase of delayed-type hypersensitivity induced by cloned helper cells. Int Arch Allergy Appl Immunol 86:102–105

Kamei C, Izushi K, Adachi Y, Shimazawa M, Tasaka K (1991) Inhibitory effect of epinastine on the type II–IV allergic reactions in mice, rats and guinea pigs. Arzneim Forsch/Drug Res 41:1150–1153

Li F, Song D, Lu Y, Zhu H, Chen Z, He X (2013) Delayed-type hypersensitivity (DTH) immune response related with EBV-DNA in nasopharyngeal carcinoma treated with autologous dendritic cell vaccination after radiotherapy. J Immunother 36:208–214

Malajian D, Belsito DV (2013) Cutaneous delayed-type hypersensitivity in patients with atopic dermatitis. J Am Acad Dermatol 69:232–237

Mizukoshi S, Tsukamoto M, Tanaka H, Nakamura K, Kato F (1994) Antiinflammatory and immunosuppressive effects of 1,6-anhydro-3,4-dideoxy-2-furfuryl-*β*-D-*threo*-3-enopyranose (MT 2221), a novel anhydro-enopyranose derivative, on experimental animal models. Biol Pharm Bull 17:1070–1074

Nagakawa Y, Ogawa T, Kobayashi M, Wagatsuma K, Munakata H, Umezu K, Sato S, Shibata Y, Inoue K, Ishida N (1990) Immunopharmacological studies of 4-acetylaminophenyl-acetic acid. (MS-932). Int J Immunother 6:131–140

Pence BD, Lowder TW, Keylock KT, Potter VJV, Cook MD, McAuley E, Woods JA (2012) Relationship between systemic inflammation and delayed-type hypersensitivity response to Candida antigen in older adults. PLoS One 7:e36403

Schindewolf M, Gobst C, Kroll H, Recke A, Louwen F, Wolter M, Kaufmann R, Boehncke W-H, Lindhoff-Last E, Ludwig RJ (2013) High incidence of heparin-induced allergic delayed-type hypersensitivity reactions in pregnancy. J Allergy Clin Immunol 132:131–139

Titus RG, Chiller JM (1981) A simple and effective method to assess murine delayed type hypersensitivity to proteins. J Immunol Methods 45:65–78

Yang H, Troudt J, Grover A, Arnett K, Lucas M, Cho YS, Bielefeldt-Ohmann H, Talor J, Izzo A, Dobos KM (2011) Three protein cocktails mediate delayed-type hypersensitivity responses indistinguishable from that elicited by purified protein derivative in the guinea pig model of *Mycobacterium tuberculosis* infection. Infect Immun 79:716–723

Reversed Passive Arthus Reaction

Bailey PJ, Sturm A (1983) Immune complexes and inflammation. A study of the activity of anti-inflammatory drugs in the reverse passive Arthus reaction in the rat. Biochem Pharmacol 32:475–481

Berkenkopf JW, Weichman BM (1991) Comparison of several new 5-lipoxygenase inhibitors in a rat Arthus pleurisy model. Eur J Pharmacol 193:29–34

Berkenkopf JW, Marinari LR, Weichman BM (1991) Phospholipase A_2 acyl-hydrolytic activity in rat RPAR-induced pleurisy. Agents Actions 34:93–96

Burch RM, Connor JR, Bator JM, Weitzberg M, Laemont K, Noronha-Blob L, Sullivan JP, Steranka LR (1992) NPC 15669 inhibits the reverse passive Arthus reaction in rats by blocking neutrophil recruitment. J Pharmacol Exp Ther 263:933–937

Camussi G, Tetta C, Bussolino F, Baglioni C (1990) Antiinflammatory peptides (antiflammins) inhibit synthesis of platelet-activating factor, neutrophil aggregation and chemotaxis, and intradermal inflammatory reactions. J Exp Med 171:913–927

Carter GW, Young PR, Albert DH, Bouska J, Dyer R, Bell RL, Summers JB, Brooks DW (1991) 5-Lipoxygenase inhibitory activity of Zileuton. J Pharmacol Exp Ther 256:929–937

Chang YH, Otterness IG (1981) Effects of pharmacologic agents on the reversed passive Arthus reaction in the rat. Eur J Pharmacol 69:155–164

Humphrey JH (1955a) The mechanism of Arthus reactions. I. The role of polymorphonuclear leukocytes and other factors in reversed passive Arthus reactions in rabbits. Br J Exp Pathol 36:268–282

Humphrey JH (1955b) The mechanism of Arthus reactions. II. The role of polymorphonuclear leukocytes and platelets in reversed passive Arthus reactions in the guinea-pig. Br J Exp Pathol 36:283–289

Kim KH, Martin IC, Young PR, Carter GW, Haviv F (1990) Inhibitors of immune complex-induced inflammation: 5-substituted 3-[1-(2-benzoxazolyl) hydrazino]propanenitrile derivatives. J Pharm Sci 79:682–684

Okamoto H, Iwahisa Y, Terawasa M (1992) Suppression of the Arthus reaction by Y-24180, a potent and specific antagonist of platelet-activating factor. Agents Actions 35:149–158

Ting PC, Kaminski JJ, Sherlok MH, Tom WC, Lee JF, Bryant RW, Watnick AD, McPhail AT (1990) Substituted 1,3-dihydro-2*H*-pyrrolo[2,3-*b*]pyridin-2-ones as potential antiinflammatory agents. J Med Chem 33:2697–2706

Yamamoto S, Dunn CD, Deporter DA, Capasso F, Willoughby DA, Huskisson EC (1975) A model for the quantitative study of Arthus (immunologic) hypersensitivity in rats. Agents Actions 5:374–377

Adjuvant Arthritis in Rats

Ajmal M (1969) Erysipelothrix rhusiopathiae and spontaneous arthritis in pigs. Res Vet Sci 10:579

Arner EC, Harris RR, DiMeo TM, Collins RC, Galbraith W (1995) Interleukin-1 receptor antagonist inhibits proteoglycan breakdown in antigen induced but not in polycation induced arthritis in the rabbit. J Rheumatol 22:1338–1346

Balague C, Pont M, Prats N, Godessart N (2012) Profiling of dihydroorotate dehydrogenase, p38 and JAK inhibitors in the rat adjuvant-induced arthritis model. Br J Pharmacol 166:1320–1332

Bartlett RR, Schleyerbach R (1985) Immunopharmacological profile of a novel isoxazol derivative, HWA 486, with potential antirheumatic activity. I. Disease modifying action on adjuvant arthritis of the rat. Int J Immunopharmacol 7:7–18

Beck FWJ, Whitehouse MW, Pearson CM (1974) Drug sensitivity of rat adjuvant arthritis, induced with 'adjuvants' containing no mineral oil components. Proc Soc Exp Biol Med 146:665–669

Boe A, Baiocchi M, Carbonatto M, Papoian R, Serlupi-Crescenzi O (1999) Interleukin 6 knock-out mice are resistant to antigen-induced experimental arthritis. Cytokine 11:1057–1064

Bolon B, Shalhoub V, Kostenuik PJ, Campagnuolo G, Morony S, Boyle WJ, Zack D, Feige U (2002a) Osteoprotegerin, an endogenous antiosteoclast factor for protecting bone in rheumatoid arthritis. Arthritis Rheum 46:3121–3135

Bolon B, Campagnuolo G, Feige U (2002b) Duration of bone protection by a single osteoprotegerin injection in rats with adjuvant-induced arthritis. Cell Mol Life Sci 59:1569–1576

Bolon B, Morony S, Cheng Y, Hu YL, Feige U (2004) Osteoclast numbers in Lewis rats with adjuvant-induced arthritis: identification of preferred sites and parameters for rapid quantitative analysis. Vet Pathol 41:30–36

Boyle DL, Kowaluk EA, Jarvis MF, Lee CH, Bhagwat SS, Williams W, Firestein GS (2001) Anti-inflammatory effects of ABT-702, a novel non-nucleoside adenosine kinase inhibitor, in rat adjuvant arthritis. J Pharmacol Exp Ther 296:495–500

Brackertz D, Mitchell GF, MacKay IR (1977) Antigen-induced arthritis in mice. Arthritis Rheum 20:841–850

Brun JG, Haland G, Haga HJ, Fagerhol MK, Jonsson R (1995) Effect of calprotectin in avridine-induced arthritis. APMIS 103:233–240

Butler SH, Godefroy F, Besson JM, Weil-Fugazza J (1991) Increase in "pain sensitivity" induced by exercise applied during the onset of arthritis in a model of monoarthritis in the rat. Int J Tissue React 13:299–304

Campagnuolo G, Bolon B, Feige U (2002) Kinetics of bone protection by recombinant osteoprotegerin therapy in Lewis rats with adjuvant arthritis. Arthritis Rheum 46:1926–1936

Cayeux P, Panijel J, Cluzan R, Levillain R (1966) Streptococcal arthritis and cardiomyopathy experimentally induced in white mice. Nature 212:688–691

Cecil RL, Angevine DM, Rothbard S (1939) Experimental arthritis in rabbits produced by streptococci and other organisms. Am J Med Sci 198:463–475

Colpaert FC (1987) Evidence that adjuvant arthritis in the rat is associated with chronic pain. Pain 28:201–222

Connolly KM, Stecher VJ, Danis E, Pruden DJ, LaBrie T (1988) Alteration of interleukin-1 production and the acute phase response following medication of adjuvant arthritic rats with cyclosporin-A or methotrexate. Int J Immunopharmacol 10:717–728

Consden R, Doble A, Glynn LE, Nind AP (1971) Production of a chronic arthritis with albumin. Its retention in rabbit knee joints. Ann Rheum Dis 30:307–315

Cook J, Fincham WJ (1966) Arthritis produced by intra-articular injection of streptolysin S in rabbits. J Pathol Bacteriol 99:283–297

Cooke TD, Jasin HE (1972) The pathogenesis of chronic inflammation in experimental antigen-induced arthritis. I. The role of antigen on the local immune response. Arthritis Rheum 15:327–337

Cooke TD, Hurd ER, Ziff M, Jasin HE (1972) The pathogenesis of chronic inflammation in experimental antigen-induced arthritis. II. Preferential localization of antigen-antibody complexes to collagenous tissues. J Exp Med 135:323–338

Crossley MJ, Holland T, Spowage M, Hunneyball IM (1989) Monarticular antigen-induced arthritis in rabbits and mice. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 415–439

Cruwys SC, Garrett NE, Perkins MN, Blake DR, Kidd BL (1994) The role of bradykinin B_1 receptors in the maintenance of intra-articular plasma extravasation in chronic antigen-induced arthritis. Br J Pharmacol 113:940–944

Cullen GA (1977) Mycoplasma infection and arthritis in chickens. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 240–255

Cuzzocrea S, Wayman NS, Mazzon E, Dugo L, di Paola R, Serraino I, Britti D, Chatterjee PK, Caputi AP, Thiemermann C (2002) The cyclopentenone prostaglandin 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 attenuates the development of acute and chronic inflammation. Mol Pharmacol 61:997–1007

Darwish SF, El-Bakly WM, Arafa HM, El-Demerdash E (2013) Targeting TNF-a and NF-kB activation by bee venom: role in suppressing adjuvant induced arthritis and methotrexate hepatotoxicity in rat. PLoS One 8: e79284

del Pozo E, Graeber M, Payne T (1990) Regression of bone and cartilage loss in adjuvant arthritic rats after treatment with cyclosporin A. Arthritis Rheum 33:247–252

Esser RE, Hildebrand AR, Angelo RA, Watts AM, Murphey MD, Baugh LE (1995) Measurement of radiographic changes in adjuvant-induced arthritis in rats by quantitative image analysis. Arthritis Rheum 38:129–138

Francischi JN, Yokoro CM, Poole S, Tafuri WL, Cunha FQ, Teixeira MM (2000) Anti-inflammatory and analgesic effects of the phosphodiesterase 4 inhibitor rolipram in a rat model of arthritis. Eur J Pharmacol 399:243–249

Fujisawa T, Igeta K, Odake S, Morita Y, Yasuda J, Morikawa T (2002) Highly-water soluble matrix metalloproteineases inhibitors and their effects in a rat adjuvant-induced arthritis model. Bioorg Med Chem 10:2569–2581

Gardner DL (1960) The experimental production of arthritis. A review. Ann Rheum Dis 19:297–317

Gauldie SD, McQueen DS, Clarke CJ, Chessell IP (2004) A robust model of adjuvant-induced chronic unilateral arthritis in two mouse strains. J Neurosci Methods 139:281–291

Ginsburg I, Silberstein Z, Spira G, Bentwich Z, Boss JH (1968) Experimental arthritis in rabbits induced by group A streptococcal products. Experientia (Basel) 24:256–257

Ginsburg I, Zor U, Floman Y (1977) Experimental models of streptococcal arthritis: pathogenic role of streptococcal products and prostaglandins and their modification by anti-inflammatory agents. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 256–299

Glynn LE (1977) Erysipelothrix arthritis in rabbits. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 238–239

Henderson B, Pettipher ER, Murphy G (1990) Metalloproteinases and cartilage proteoglycan depletion in chronic arthritis. Comparison of antigen-induced and polycation-induced arthritis. Arthritis Rheum 33:241–246

Hook EW, Wagner RR, Lancefield RC (1960) An epizootic in Swiss mice caused by a group A streptococcus, newly designed type 50. Am J Hyg 72:11–119

Issekutz AC, Meager A, Otterness I, Issekutz TB (1994) The role of tumor necrosis factor-alpha and IL-1 in polymorphonuclear leukocyte and T lymphocyte recruitment to joint inflammation in adjuvant arthritis. Clin Exp Immunol 97:26–32

Jasin HE, Cooke TD (1977) Persistence of antigen in experimental allergic monoarthritis. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/Heidelberg/New York, pp 28–32

Jasmin G (1967) Experimental arthritis in rats. A comprehensive review with specific reference to mycoplasma. In: Rohstein J (ed) Rheumatology, vol 1. Karger, Basel, pp 107–131

Joe B, Wilder RL (1999) Animal models of rheumatoid arthritis. Mol Med Today 5:367–369

Kawahito Y, Kondo M, Tsubouchi Y, Hashiramoto A, Bishop-Bailey D, Inoue KI, Kohno M, Yamada R, Hla T, Sano H (2000) 15-deoxy-$\Delta^{12,14}$-PGJ_2 induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. J Clin Invest 106:189–197

Kazuna S, Kawai K (1975) Evaluation of analgesic agents in rats with adjuvant arthritis. Chem Pharm Bull (Tokyo) 23:1184–1191

Kerr KM, Olson NO (1970) Pathology of chickens inoculated experimentally or contact-infected with *Mycoplasma synoviae*. Avian Dis 14:291–320

Kiely PDW, Thiru S, Oliveira DGB (1995) Inflammatory polyarthritis by mercuric chloride in the Brown Norway rat. Lab Invest 73:284–293

Kiely PDW, O'Brien D, Oliveira DGB (1996) Anti-CD8 treatment reduces the severity of inflammatory arthritis,

but not vasculitis, in mercuric chloride-induced autoimmunity. Clin Exp Immunol 106:280–285
Kim EY, Moudgil KD (2009) The determinants of susceptibility/resistance to adjuvant arthritis in rats. Arthritis Res Ther 11:239–248
Koga T, Pearson CM, Narita T, Kotani S (1973) Polyarthritis induced in the rat by cell walls from several bacteria and two Streptomyces species. Proc Soc Exp Biol N Y 143:824–827
Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li JI, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM (1999) Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402:304–309
Leisten JC, Gaarde WA, Scholz W (1990) Interleukin-6 serum levels correlate with footpad swelling in adjuvant-induced arthritic Lewis rats treated with cyclosporin A or indomethacin. Clin Immunol Immunopathol 56:108–115
Lewis EJ, Bishop J, Bottomley KM, Bradshaw D, Brewster M, Broadhurst MJ, Brown PA, Budd JM, Elliott L, Greenham AK, Johnson WH, Nixon JS, Rose F, Sutton B, Wilson K (1997) Ro 32–3555, an orally active collagenase inhibitor, prevents cartilage breakdown in vitro and in vivo. Br J Pharmacol 121:540–546
Lewthwaite J, Blake S, Thompson RC, Hardingham TE, Henderson B (1995) Antifibrotic action of interleukin-1 receptor antagonist in lapine monoarticular arthritis. Ann Rheum Dis 54:591–596
Lorentzen JC, Klareskog L (1997) Comparative susceptibility of DA, LEW, and LEW.1AV1 rats to arthritis induced by different arthritogens: mineral oil, mycobacteria, muramyl dipeptide avridine and rat collagen II. Transplant Proc 29:1692–1693
Meacock SC, Brandon DR, Billigham ME (1994) Arthritis in Lewis rats induced by the non-immunogenic adjuvant CP20961: an immunohistochemical analysis of the developing disease. Ann Rheum Dis 53:653–658
Mohr W, Wild A (1976) Adjuvant arthritis. Arzneim Forsch/Drug Res 26:1860–1866
Moran EL, Bogoch ER (1999) Animal models of rheumatoid arthritis. In: An YH, Friedman RJ (eds) Animal models in orthopaedic research. CRC Press LLC, Boca Raton, pp 369–390
Norlin G (1960) Experimental rheumatoid arthritis in rabbits. Acta Rheumatol Scand 6:309–319
Ohanian SH, Schwab JH, Cromartie WJ (1969) Relation to rheumatic-like lesions of the mouse to localization of group A streptococci cell walls. J Exp Med 129:37–49
Olson NO, Bletner JK, Shelton DC, Munro DA, Anderson GC (1954) Enlarged joint condition in poultry caused by an infectious agent. Poultry Sci 33:1075–1080
Olson NO, Kerr KM, Cambell A (1964) Control of infectious synovitis. The antigen study of three strains. Avian Dis 8:209–215
Pearson CM (1956) Development of arthritis, periarthritis and periostitis in rats given adjuvants. Proc Soc Exp Biol Med 91:95–101
Pearson CM (1963) Experimental joint disease. Observations on adjuvant-induced arthritis. J Chronic Dis 16:863–874
Pearson CM, Wood FD (1959) Studies on polyarthritis and other lesions induced in rats by injection of mycobacterium adjuvant. I. General clinic and pathological characteristics and some modifying factors. Arthritis Rheum 2:440–459
Perper RJ, Alvarez B, Colombo C, Schroder H (1971) The use of a standardized adjuvant arthritis assay to differentiate between anti-inflammatory and immunosuppressive agents. Proc Soc Exp Biol Med 137:506–512
Pircio AW, Fedele CT, Bierwagen ME (1975) A new method for the evaluation of analgesic activity using adjuvant-induced arthritis in the rat. Eur J Pharmacol 31:207–215
Roberts ED, Ramsey KF, Switzer WP, Layton JM (1968) Pathologic changes of porcine suppurative arthritis produced by *Streptococcus equisimilis*. Am J Vet Res 29:253–262
Roberts ED, Ramsey KF, Switzer WP, Layton JM (1969) Electron microscopy of porcine synovial membrane cell layer in *Streptococcus equisimilis* arthritis. J Comp Pathol 79:47–51
Rooks WH, Tomolonis AJ, Maloney PJ, Wallach MB, Schuler ME (1982) The analgesic and anti-inflammatory profile of (± − 5-benzoyl-1,2-dihydro-3H-pyrrolo[1,2a]pyrrole-1-carboxylic acid (RS-37619). Agents Actions 12:684–690
Sandow J, Alpermann H, Metzger H, Vogel HG (1971) α-2-Glycoprotein levels in the experimental immunoarthritis of the rat. Naunyn-Schmiedeberg's Arch Pharmacol 269:483
Schorlemmer HU, Dickneite G (1992) Preclinical studies with 15-deoxyspergualin in various animal models for autoimmune diseases. Ann N Y Acad Sci 685:155–174
Schorlemmer HU, Kurrle R, Schleyerbach R, Bartlett RR (1999) Disease-modifying activity of malononinitrilamides, derivates of leflunomide's active metabolite, on models of rheumatoid arthritis. Inflamm Res 48 (Suppl 2):S113–S114
Schulz LC, Drommer W, Seidler D, Ehard H, Mickwitz G, Hertrampf B, Böhm KH (1975a) Experimenteller Rotlauf bei verschiedenen Spezies als Ursache einer systemischen Bindegewebskrankheit. I. Systemische vaskuläre Prozesse bei der Organmanifestation. Beitr Path 154:1–20
Schulz LC, Drommer W, Seidler D, Ehard H, Leimbeck R, Weiss R (1975b) Experimenteller Rotlauf bei verschiedenen Spezies als Modell einer systemischen Bindegewebskrankheit. II. Chronische Phase mit besonderer Berücksichtigung der Polyarthritis. Beitr Pathol 154:27–51
Schulz LC, Ehard H, Hertrampf B, Drommer W, Seidler D, Böhm KH (1977) Hemostasis, fibrin incorporation and local mesenchymal reaction in erysipelothrix infection

as a model for rheumatism research. In: Glynn LE, Schlumberger HD (eds) Experimental models of chronic inflammatory diseases. Springer, Berlin/ Heidelberg/New York, pp 215–237

Shi X-L, Wang L-P, Feng X, Fan D-D, Zang W-J, Wang B, Zhou J (2014) Inhibition of adjuvant-induced arthritis by nasal administration of novel synthetic peptides from heat shock protein 65. BMC Muskuloskelet Disord 15:252. doi: 10.1186/1471-2474-15-253

Shimizu G, Shichikawa K, Takiuchi K (1958) Studies on the etiology of rheumatic arthritis. III. Experimental production of arthritis in rabbits following focal infection of paranasal sinus with hemolytic streptococcus. Med J Osaka Univ 9:447–468

Snekhalatha U, Anburajan M, Venkatraman B, Menaka M (2013) Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model. Zeitschrift Rheumatol 72:375–388

Stein H, Yarom R, Levine S, Dishon T, Ginsburg I (1973) Chronic self-perpetuating arthritis induced in rabbits by a cell-free extract of group A streptococci. Proc Soc Exp Biol N Y 143:1106–1112

Tsurumi K, Kokuba S, Okada K, Yanagihara M, Fujimura H (1986) Pharmacological investigations of the new antiinflammatory agent 2-(10,11-dihydro-10-oxodibenzo[b, f]thiepin-2-yl) propionic acid. 4th communication: inhibitory effects on rat adjuvant arthritis. Arzneim-Forsch/Drug Res 36:1810–1817

Van Bilsen JHM, Wagenaar-Hilbers JPA, Grosfeld-Stulemeijer MCJT, van der Cammen MJF, van Dijk MEA, van Eden E, Wauben MHM (2004) Matrix metalloproteinases as targets for the immune system during experimental arthritis. J Immunol 172:5063–5068

Vingsbo C, Jonsson R, Holmdahl R (1995) Avridine-induced arthritis in rats: a T cell-dependent chronic disease influenced both by MHC genes and by non-MHC genes. Clin Exp Immunol 99:359–363

Vollmer S, Gemeinhardt I, Vater A, Schnorr B, Schnorr J, Voigt J, Ebert B (2014) In vivo therapy monitoring of experimental rheumatoid arthritis in rats using near-infrared fluorescence imaging. J Biomed Opt 19. doi: 10.1117/1.JBO.19.3.036011

Wei YH, Li Y, Qiang CJ (2004) Effects and mechanisms of FR167653, a dual inhibitor of interleukin-1 and tumor necrosis factor, on adjuvant arthritis in rats. Int Immunopharmacol 4:1625–1632

Walz DT, DiMartino MJ, Kuch JH, Zuccarello W (1969) Adjuvant-induced arthritis in rats – temporal relationship of drug effects on physiological, biochemical, and haematological parameters. Pharmacologist 11:266

Wang B (2014) Anti-arthritic effect of astragaloside IV and its molecular mechanism. Inflamm Cell Signal 1:e130

Weichman BM (1989) Rat adjuvant arthritis: a model of chronic inflammation. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 363–380

White TG, Jl P, Hargrave P (1975) Production of synovitis in rabbits by fractions of a cell-free extract of *Erysipelothrix rhusiopathiae*. Clin Immunol Immunopathol 3:531–540

Wilder RL, Calandra GB, Garvin AJ, Wright KD, Hansen CT (1982) Strain and sex variation in the susceptibility to streptococcal wall-induced polyarthritis in the rat. Arthritis Rheum 25:1064–1072

Wilder RL, Allen JB, Hansen C (1987) Thymus-dependent and -independent regulation of Ia antigen expression *in situ* by cells in the synovium of rats with streptococcal cell wall-induced arthritis. J Clin Invest 79:1160–1171

Yocum DE, Allen JB, Wahl SM, Calandra GB, Wilder RL (1986) Inhibition by cyclosporin A of streptococcal wall-induced arthritis and hepatic granulomas in rats. Arthritis Rheum 29:262–273

Zhang Z-C, Zhang S-J, Jin B, Wu Y, Yang X-F, Yu B, Xie Q-M (2014) Ciclamilast ameliorates adjuvant-induced arthritis in a rat model. BioMed Res Int. Article ID 786104 (in press)

Collagen Type II Induced Arthritis in Rats

Anthony Balducci A, Helfer BM, Ahrens ET, O'Hanlon III CF, Wesa AK Visualizing arthritic inflammation and therapeutic response by fluorine-19 magnetic resonance imaging (^{19}F MRI). J Inflamm 9:24

Balducci A, Helfer BM, Ahrens ET, O'Hanlon CF, Wesa AK (2012) Visualizing arthritic inflammation and therapeutic response by fluorine-19 magnetic resonance imaging (19F MRI). J Inflamm 9:24. doi:10.1186/1476-9255-9-24

Bevaart L, Vervoordeldonk MJ, Tak PP (2010) Evaluation of therapeutic targets in animal models arthritis. Arthritis Rheum 62:2192–2205

Bolon B, Stolina M, King C, Middleton S, Gasser J, Zack DU (2011) Rodent preclinical models for developing novel antiarthritic molecules: comparative biology and preferred methods for evaluating efficacy. J Biomed Biotechnol 2011. Article ID 569068, 21 pp. doi:10.1155/2011/569068

Cannon GW, McCall S, Cole BC, Griffiths MM, Radov LA, Ward JR (1990) Effects of indomethacin, cyclosporin, cyclophosphamide, and placebo on collagen-induced arthritis of mice. Agents Actions 29:315–323

Carlson RP, Baeder WL, Caccese RG, Warner LM, Sehgal SN (1992) Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. Ann N Y Acad Sci 685:86–113

Chen JJ, Dewdney N, Lin X, Martin RL, Walker KAM, Huang J, Chu F, Eugui E, Mirkovich A, Kim Y, Sarma K, Arzeno H, van Wart HE (2003) Design and synthesis of orally active inhibitors of TNF synthesis as anti-rheumatoid arthritis drugs. Bioorg Med Chem Lett 13:3951–3954

Cuzzocrea S, Mazzon E, Dugo L, Patel NSA, Seraino I, di Paola R, Genovese T, Britti D, de Maio M, Caputi AP, Theimermann C (2003) Reduction in the evolution of

murine type II collagen-induced arthritis by treatment with rosiglitazone, a ligand of the peroxisome proliferator-activated receptor γ. Arthritis Rheum 48:3544–3556

Fujii Y, Hirayama T, Ohtake H, Ono N, Inoue T, Sakurai T, Takayama T, Matsumoto K, Tsukahara N, Hidano S, Harima N, Nakazawa K, Igarashi Y, Goitsuka R (2012) Amelioration of collagen-induced arthritis by a novel SIP_1 antagonist with immunomodulatory activities. J Immunol 188:206–215

Hegen M, Keith JC Jr, Collins M, Nickerson-Nutter CL (2008) Utility of animal models for identification of potential therapeutics for rheumatoid arthritis. Ann Rheum Dis 67:1505–1515

Henderson H, Staines NA, Burrai I, Cox JH (1984) The anti-arthritic and immunosuppressive effects of cyclosporine on arthritis induced in the rat by type II collagen. Clin Exp Immunol 57:51–56

Hom JT, Butler LD, Riedl PE, Bendele AM (1988) The progression of the inflammation in established collagen-induced arthritis can be altered by treatments with immunological or pharmacological agents which inhibit T cell activities. Eur J Immunol 18:881–888

Hu Y, Liu R, Li J, Yue Y, Cheng W, Zhang P (2014) Attenuation of collagen-induced arthritis in rat by nicotinic alpha7 receptor partial agonist GTS-21. BioMed Res Int 2014. Article ID 325875, 9 pp. doi:10.1155/2014/325875

Janusz MJ, Durham SL (1997) Inhibition of cartilage degradation in rat collagen-induced arthritis but not adjuvant arthritis by the neutrophil elastase inhibitor MDL 101,146. Inflamm Res 46:503–508

Joosten LAB, Helsen MMA, Van den Berg WB (1994) Accelerated onset of collagen-induced arthritis by remote inflammation. Clin Exp Immunol 97:204–211

Joosten LAB, Helsen MMA, Saxne T, van de Loo FAJ, Heinegård D, van den Berg WB (1999) IL-1$\alpha\beta$ blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-α blockade only ameliorates joint inflammation. J Immunol 163:5049–5055

Kaibara N, Hotokebuchi T, Takagishi K, Katsuki I (1983) Paradoxical effects of cyclosporin A on collagen arthritis in rats. J Exp Med 158:2007–2015

Kumar V, Aziz F, Sercarz E, Miller A (1997) Regulatory T cells specific for the same framework 3 region of the Vβ8.2 chain are involved in the control of collagen II-induced arthritis and experimental autoimmune encephalomyelitis. J Exp Med 185:1725–1733

Kuno M, Seki N, Tsujimoto S, Nakanishi I, Kinoshita T, Nakamura K, Terasaka T, Nishio N, Sato A, Fujii T (2006) Anti-inflammatory activity of non-nucleoside adenosine deaminase inhibitor FR234938. Eur J Pharmacol 534:241–249

Levine YA, Koopman FA, Faltys M, Caravaca A, Bendele A, Zitnik R, Vervoordeldonk MJ, Tak PP (2014) Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. PLoS One. doi: 10.1371/journal.pone.0104530

Liu L, di Paolo J, Barbosa J, Rong H, Reif K, Wong H (2011) Antiarthritis effect of a novel Bruton's tyrosine kinase (BTK) inhibitor in rat collagen-induced arthritis and mechanism-based pharmacokinetic/pharmacodynamic modeling: relationships between inhibition of BTK phosphorylation and efficacy. J Pharmacol 338:154–163

Lubberts E, Koenders MI, Oppers-Walgreen B, van den Bersselaar L, Coenen-de Roo CJJ, Joosten LAB, van den Berg WB (2004) Treatment with a neutralizing anti-murine interleukin-17 antibody after the onset of collagen-induced arthritis reduces joint inflammation, cartilage destruction, and bone erosion. Arthritis Rheum 50:650–659

McIntyre KW, Shuster DJ, Gillooly KM, Dambach DM, Pattoli MA, Lu P, Zhou XD, Zusi FC, Burke JR (2003) A highly selective inhibitor of IκB kinase, BMS-.345541, blocks both inflammation and destruction in collagen-induced arthritis in mice. Arthritis Rheum 48:2652–2659

Miesel R, Haas R (1993) Reactivity of an active center analog of Cu_2Zn_2-superoxide dismutase in murine model of acute and chronic inflammation. Inflammation 17:595–611

Miesel R, Dietrich A, Brandl B, Ulbrich N, Kurpisz M, Kröger H (1994a) Suppression of arthritis by an active center analogue of Cu_2Zn_2-superoxide dismutase. Rheumatol Int 14:119–126

Miesel R, Kröerg H, Ulbrich N, Kurpisz M (1994b) Arthritogenic reactivity of chromium (V). Z Rheumatol 53:59

Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, Sekikawa K, Asano M, Iwakura Y (2002) Antigen-specific T cell sensitization in impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 17:375–378

Nakae S, Nambu A, Sudo K, Iwakura Y (2003) Suppression of immune induction of collagen-induced arthritis in IL-17-deficient mice. J Immunol 171:6173–6177

Nemoto K, Mae T, Abe F, Takeuchi T (1992) Successful treatment with a novel immunosuppressive agent, deoxyspergualin, in type II collagen-induced arthritis in mice. Ann N Y Acad Sci 685:148–154

Phadke K, Carroll J, Nanda S (1982) Effects of various anti-inflammatory drugs on type II collagen-induced arthritis in rats. Clin Exp Immunol 47:579–586

Plater-Zyberg C, Joosten LAB, Helsen MMA, Sattonnet-Roche P, Siegfried C, Alouani S, van de Loo FAJ, Graber P, Aloni S, Cirillo R, Lubberts E, Dinarello CA, van den Berg WB, Chvatchko Y (2001) Therapeutic effect of neutralizing endogenous IL-18 activity in the collagen-induced model of arthritis. J Clin Invest 108:1825–1832

Podolin PL, Callahan JF, Bolognese BJ, Li YH, Carlson K, Davis TG, Mellor GF, Evans C, Roshak AK (2005) Attenuation of murine collagen-induced arthritis by a novel, potent, selective small molecule inhibitor of IκB

kinase 2, TPCA-1 (2[(aminocarbonyl) amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide), occurs via reduction of proinflammatory cytokines and antigen-induced T cell proliferation. J Pharmacol Exp Ther 312:373–381

Romas E, Sims NA, Hards DK, Lindsay M, Quinn JWM, Ryan OFJ, Dunstan CR, Martin TJ, Gillespie MT (2002) Osteoprotegerin reduces osteoclast numbers and prevents bone erosion in collagen-induced arthritis. Am J Pathol 161:1419–1427

Roy T, Ghosh S (2013) Animal models of rheumatoid arthritis: correlation and usefulness with human rheumatoid arthritis. Indo Am J Pharm Res 3:6131–6142

Ruchatz H, Leung BP, Wi XQ, McInnes IB, Liew FY (1998) Soluble IL-15 receptor α-chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology. J Immunol 160:5654–5660

Probeert AW, Schrier DJ, Gilbertsen RB (1984) Effects of anti-arthritic compounds on type II collagen-induced arthritis in rats. Arch Int Pharmacodyn Ther 269:167–176

Sevilla RS, Cruz F, Chiu C-S, Xue D, Bettano KA, Zhu J, Chakravarthy K, Faltus R, Wang S, Vanko A, Robinson G, Zielstorff M, Miao J, Leccese E, Conway D, Moy LY, Dogdas B, Cicmil M, Zhang W (2015) Development and optimization of a high throughput micro-computed tomography imaging method incorporating a novel analysis technique to evaluate bone mineral density of arthritic joints in a rodent model of collagen induced arthritis. Bone 73:32–41

Takagishi K, Kaibara N, Hotokebuchi T, Arita C, Morinaga M, Arai K (1986) Effects of cyclosporin on collagen induced arthritis in mice. Ann Rheum Dis 45:339–344

Takagishi K, Yamamoto M, Miyahara H, Hotokebuchi T, Kaibara N (1992) Comparative study of effects of cyclosporins A and G on collagen arthritis in mice. Agents Actions 37:284–289

Tanaka K, Shimotori T, Makino S, Aikawa Y, Inaba T, Yoshida C, Takano S (1992) Pharmacological studies of the new anti-inflammatory agent 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one. 1st communication: anti-inflammatory, analgesic and other related properties. Arzneim Forsch/Drug Res 42:935–944

Trentham DE, Dynesius-Trentham RA (1989) Type II collagen-induced arthritis in the rat. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 395–413

Trentham DE, Townes AS, Kang AH (1977) Autoimmunity to type II collagen: an experimental model of arthritis. J Exp Med 146:857–868

Wooley PH, Whalen JD, Chapman DL, Berger AE, Richard KA, Aspar DG, Staite ND (1993) The effect of an interleukin-1 receptor antagonist protein on type II collagen-induced arthritis and antigen-induced arthritis in mice. Arthritis Rheum 36:1305–1314

Proteoglycan-Induced Progressive Polyarthritis in Mice

Delemarre EM, Roord STA, van den Broek T, Zonneveld-Huijssoon E, de Jager W, Rozemuller H, Martens AC, Broere F, Wulffraat NM, Glant TT, Prakken BJ, van Wijk F (2014) Autologous stem cell transplantation in experimental arthritis by renewal and modulation of the Teff cell compartment. Arthritis Rheum 66:350–356

Glant TT, Mikecz K, Arzoumanian A, Poole AR (1987) Proteoglycan-induced arthritis in BALB/c mice: clinical features and histopathology. Arthritis Rheum 30:201–212

Glant TT, Mikecz K, Bartlett RR, Deák F, Thonar EJMA, Williams JM, Mattar T, Kuettner KE, Schleyerbach R (1992) Immunomodulation of proteoglycan-induced progressive polyarthritis by leflunomide. Immunopharmacology 23:105–116

Glant TT, Radacs M, Nagyeri G, Olasz K, Laszio A, Boldizsar F, Hegyi A, Finnegan A, Mikecz K (2011) Proteoglycan (PG)- induced arthritis (PGIA) and recombinant human PG-G1 domain-induced arthritis (GIA) in BALB/c mice resembling two subtypes of rheumatoid arthritis. Arthritis Rheum 63:1312–1321

Hascall VC, Heinegård D (1974) Aggregation of proteoglycans. I. The role of hyaluronic acid. J Biol Chem 249:4232–4241

Heinegård D (1972) Extraction, fractionation and characterization of proteoglycans from bovine tracheal cartilage. Biochim Biophys Acta 285:181–192

Mikecz K, Glant TT, Poole AR (1987) Immunity to cartilage proteoglycans in BALB/c mice with progressive polyarthritis and ankylosing spondylitis induced by injection of human cartilage proteoglycan. Arthritis Rheum 30:306–318

Mikecz K, Glant TT, Bukás E, Poole AR (1990) Proteoglycan-induced polyarthritis and spondylitis adoptively transferred to naive (nonimmunized) BALB/c mice. Arthritis Rheum 33:866–876

Poole AR (1989) Cartilage proteoglycan-induced arthritis: a combined model for rheumatoid arthritis and ankylosing spondylitis. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 441–447

Stimpson AS, Schwab JH (1989) Chronic remittent erosive arthritis induced by bacterial peptidoglycan-polysaccharide structures. In: Pharmacological methods in the control of inflammation. Alan R Liss, New York, pp 381–394

Swart JF, de Roock S, Hofhuis FM, Rozemuller H, van den Broek T, Moerer P, Broere F, van Wijk F, Kuis W, Prakken BJ, Martens ACM, Wulffraat NM (2014) Mesenchymal stem cell therapy in proteoglycan induced arthritis. Ann Rheum Dis. doi: 10.1136/annrheumdis-2013-204147

Pristane-Induced Arthritis in Mice

Abe C, Hirano S, Wakazono K, Mase T, Yamamoto R, Matsufuji M, Sakata N, Agata N, Iguchi H, Ishizuka M (1995) Effects of cytogenin on spontaneous arthritis in MRL/1 mice and on pristane-induced arthritis (PIA) in DBA/1J mice. Int J Tissue React 17:175–180

Bedwell AE, Elson CJ, Hinton CE (1987) The immunological involvement in the pathogenesis of pristane-induced arthritis. Scand J Immunol 25:393–398

Brenner M, Meng HC, Yarlett NC, Joe B, Griffiths MM, Remmers EF, Wilder RL, Gulko PS (2005) The non-MHC quantitative trait locus *Cia5* contains three major arthritis genes that differentially regulate disease severity, pannus formation, and joint damage in collagen- and pristane-induced arthritis. J Immunol 174:7894–7903

Brenner M, Braun C, Oster M, Gulka PS (2006) Thermal signature analysis as a novel method for evaluating inflammatory arthritis activity. Ann Rheum Dis 65:306–311

Chae BS, Park JS, Shin TY (2006) Endotoxin induces late increase un the production of pulmonary proinflammatory cytokines in murine lupus-like pristane-primed models. Arch Pharm Res 29:302–309

Chapdelaine JM, Whalen JD, Wooley PH (1991) Pristane-induced arthritis. II. Genetic regulation in F1 hybrid mice and cellular abnormalities following pristane injection. Autoimmunity 8:215–220

De Franco M, Peters LC, Correa MA, Galvan A, Canhamero T, Borrego A, Jensen A, Goncalves J, Cabrera WHK, Starobinas N, Ribeiro OG, Dragani T, Ibanez OM (2014) Pristane-induced arthritis loci interact with the Slc11a1 gene to determine susceptibility in mice selected for high inflammation. PLoS One. doi: 10.1371/journal.pone.0088302

Ghoraishian M, Elson CJ, Thompson SJ (1993) Comparison between the protective effects of mycobacterial 65-kD heat shock protein and ovomucoid in pristane-induced arthritis. relationship with agalactosyl IgG. Clin Exp Immunol 94:247–251

Holmberg J, Tuncel J, Yamada H, Lu S, Olofsson P, Holmdahl R (2006) Pristane, a non-antigenic adjuvant induces MHC class II-restricted, arthritogenic T cells in the rat. J Immunol 176:1172–1179

Hopkins SJ, Freemont AJ, Jayson MI (1984) Pristane-induced arthritis in BALB/c mice. I. Clinical and histological features of the arthropathy. Rheumatol Int 5:21–28

Jensen JR, Peters LC, Borrego A, Ribeiro OG, Cabrera WHK, Starobinas M, Siqueira M, Ibañez OCM, de Franco M (2006) Involvement of antibody trait loci in the susceptibility to pristane-induced arthritis in the mouse. Genes Immun 7:44–50

Lange F, Bajtner E, Rintisch C, Nandakumar KS, Sack U, Holmdahl R (2005) Methotrexate ameliorates T cell dependent autoimmune arthritis and encephalomyelitis but not antibody induced or fibroblast induced arthritis. Ann Rheum Dis 64:599–605

Levitt NG, Fernandez-Madrid F, Wooley PH (1992) Pristane induced arthritis in mice. IV. Immunotherapy with mononuclear antibodies directed against lymphocyte subsets. J Rheumatol 19:1342–1347

Lin L, Gerth AJ, Peng SL (2004) Susceptibility of mast cell-deficient W/Wv mice to pristane-induced experimental lupus nephritis. Immunol Lett 91:93–97

Lu S, Nordquist N, Holmberg J, Olofsson P, Pettersson U, Holmdahl R (2002) Both common and unique susceptibility genes in different rat strains with pristane-induced arthritis. Eur J Hum Genet 10:475–483

Morgan R, Wu B, Song Z, Wooley PH (2004) Immune reactivity to connective tissue antigens in pristane-induced arthritis. J Rheumatol 31:1497–1505

Nishikaku F, Aono S, Koga Y (1994) Protective effects of D-penicillamine and a thiazole derivative, SM-8849, on pristane-induced arthritis in mice. Int J Immunopharmacol 16:91–100

Olofsson P, Holmberg J, Pettersson U, Holmdahl R (2003) Identification and isolation of dominant susceptibility loci for pristane-induced arthritis. J Immunol 171:407–416

Patten C, Bush K, Rioja I, Morgan R, Wooley P, Trill J, Life P (2004) Characterization of pristine-induced arthritis, a murine model of chronic disease. Response to antirheumatic agents, expression of joint cytokines, and immunopathology. Arthritis Rheum 50:3334–3345

Potter M, Wax JS (1981) Genetics of susceptibility of pristine-induced plasmacytomas in BALB/sAn: reduced susceptibility in BALB/cJ with a brief description of pristine-induced arthritis. J Immunol 127:1591–1595

Richards HB, Satoh M, Shaw M, Libert C, Poli V, Reeves WH (1998) Interleukin 6 dependence of anti-DNA antibody production: evidence for two pathways of autoantibody formation in pristane-induced lupus. J Exp Med 188:985–990

Satoh M, Kumar A, Kanwar YS, Reeves WH (1995) Anti-nuclear antibody production and immune-complex glomerulonephritis in BALB/c mice treated with pristane. Proc Natl Acad Sci U S A 92:10934–10938

Stasiuk LM, Ghoraishian M, Elson CJ, Thompson SJ (1997) Pristane-induced arthritis is $CD4^+$ T cell dependent. Immunology 90:81–86

Thompson SJ, Rook GA, Brealey TJ, van der Zee R, Elson CJ (1990) Autoimmune reactions to heat-shock proteins in pristane-induced arthritis. Eur J Immunol 20:2479–2484

Thompson SJ, Francis JN, Siew LK, Webb GR, Jenner PJ, Colston MJ, Elson CJ (1998) An immunodominant epitope from mycobacterial 65-kDa heat shock protein protects against pristane-induced arthritis. J Immunol 160:4628–4634

Vigar ND, Cabrera WH, Araujo LM, Ribeiro OG, Ogata TR, Siqueira M, Ibanez OM, de Franco M (2000) Pristane-induced arthritis in mice selected for maximal or minimal acute inflammatory reaction. Eur J Immunol 30:431–437

Vingsbo C, Sahlstrand P, Brun JG, Jonsson R, Saxne T, Holmdahl R (1996) Pristane-induced arthritis in rats: a new model for rheumatoid arthritis with a chronic disease course influenced by major histocompatibility complex and non-major histocompatibility complex genes. Am J Pathol 149:1675–1683

Webster L, Olofsson P, Ibrahim SM, Holmdahl R (2003) Chronicity of pristane-induced arthritis in rats is controlled by genes on chromosome 14. J Autoimmun 21:305–313

Wooley PH, Whalen JD (1991) Pristane-induced arthritis in mice. III Lymphocyte phenotypic and functional abnormalities precede the development of pristine-induced arthritis. Cell Immunol 138:251–259

Wooley PH, Seibold JR, Whalen JD, Chapedelaine JM (1989) Pristane-induced arthritis: the immunological and genetic features of an experimental murine model of autoimmune disease. Arthritis Rheum 32:1022–1030

Wooley PH, Sud S, Whalen JD, Nasser S (1998) Pristane-induced arthritis in mice. V. Susceptibility to pristine-induced arthritis is determined by the genetic regulation of the T cell repertoire. Arthritis Rheum 41:2022–2031

Zheng CL, Hossain MA, Kukita A, Ohki K, Satoh T, Kohashi O (2002) Complete Freund's adjuvant suppresses the development and progression of pristane-induced arthritis in rats. Clin Immunol 103:204–208

Zheng CL, Ohki K, Hossain MA, Kukita A, Satoh T, Kohashi O (2003) Complete Freund's adjuvant promotes the increase of IFN-γ and nitric oxide in suppressing chronic arthritis induced by pristane. Inflammation 27:247–255

Streptococcal Cell Wall-Induced Arthritis

Chakravathy K, Faltus R, Robinson G, Sevilla R, Shin J, Zielstorff M, Byford A, Leccese E, Caniga MJ, Hseih S, Zhang S, Chiu C-S, Zhang-Hoover J, Moy LY, McLeod RL, Stoffregen D, Zhang W, Murtaza A, Cicmil M (2014) Etanercept ameliorates inflammation and pain in a novel mono-arthritic multi-flare model of streptococcal cell wall induced arthritis. BMC Musculoskel Disord 15:409. doi: 10.1186/1471-2474-15-409

Cromartie WJ, Craddock JG, Schwab JH, Anderle SK, Yang C-H (1977) Arthritis in rats after systemic injection of streptococcal cells or cell walls. J Exp Med 146:1585–1602

Kuiper S, Joosten LAB, Bendele AM, Edwards CK III, Arntz OJ, Helsen MMA, van de Loo FAJ, van den Berg WB (1998) Different roles of tumor necrosis factor α and interleukin 1 in murine streptococcal cell wall arthritis. Cytokine 10:690–702

Laemont KD, Schaefer CJ, Juneau PL, Schrier DJ (1999) Effects of the phosphodiesterase inhibitor rolipram on streptococcal cell wall-induced arthritis in rats. Int J Immunopharmacol 21:711–725

McInnes IB, Cruwys S, Bowers K, Braddock M (2014) Targeting the $P2X_7$ receptor in rheumatoid arthritis: biological rationale for $P2X_7$ antagonism. Clin Exp Rheumatol 32:878–882

Richards PJ, Williams BD, Williams AS (2001) Suppression of chronic streptococcal cell wall-induced arthritis in Lewis rats by liposomal clodronate. Rheumatology 40:978–987

Rioja I, Clayton CL, Graham SJ, Life PF, Dickson MC (2005) Gene expression profiles in the rat streptococcal cell wall-induced arthritis model identified using microarray analysis. Arthritis Res Ther 7:R101–17

Schimmer RC, Schrier DJ, Flory CM, Laemont KD, Tung D, Metz AL, Friedl HP, Conroy MC, Warren JS, Beck B, Ward PA (1998) Streptococcal cell wall-induced arthritis: requirements for IL-4, IL-10, IFN-γ and monocyte chemoattractant protein-1. J Immunol 160:1466–1471

Schrier DJ, Schimmer RC, Flory CM, Tung DK-L, Ward PA (1998) Role of chemokines and cytokines in a reactivation model of arthritis induced by injection with streptococcal cell walls. J Leukoc Biol 63:359–363

Van den Broek MF, van den Berg WB, van de Putte LBA, Severijnen AJ (1988) Streptococcal cell wall-induced arthritis and flare-up reaction in mice induced by homologous or heterologous cell walls. Am J Pathol 133:139–149

Van den Broek MF, de Heer E, van Bruggen MCJ, de Roo G, Kleiverda K, Eulderink F, van den Berg WB (1992) Immunomodulation of streptococcal cell wall-induced arthritis. Identification of inflammatory cells and regulatory T cell subsets by mercuric chloride and in vivo CD8 depletion. Eur J Immunol 22:3091–3095

Wang SX, Cherian A, Dumitriu M, Grynpas MD, Carran J, Wainman D, Anastassiades T (2007) Disease modifying effects of *N*-butyryl glucosamine in a streptococcal cell wall induced arthritis model in rats. J Rheumatol 34:712–720

Yocum DE, Allen JB, Wahl SM, Calandra GB, Wilder RL (1986) Inhibition by cyclosporin A of streptococcal cell wall-induced arthritis and hepatic granulomas in rats. Arthritis Rheum 29:262–273

Experimental Autoimmune Thyroiditis

Castagliola S, Many MC, Stalmans-Falys M, Tonacchera M, Vassart G, Ludgate M (1994) Recombinant thyrotropin receptor and the induction of autoimmune thyroid disease in BALB/c mice: a new animal model. Endocrinology 135:2150–2159

Castagliola S, Many MC, Stalmans-Falys M, Vassart G, Ludgate M (1996) Transfer of thyroiditis, with syngeneic spleen cells sensitized with the human thyrotropin

receptor, to naive BALB/c and NOD mice. Endocrinology 137:4637–4643
Fang Y, Zhao L, Parker CA, Yan F, Zhang C (2010) Modulation of apoptosis: new opportunities for drug discovery to treat autoimmune thyroiditis. Recent Pat Inflamm Allergy Drug Discov 4:255–260
Fournier C, Gepner P, Saouk M, Charreire J (1990) In vivo beneficial effects of cyclosporin A and 1,25-dihydroxyvitamin D_3 on the induction of experimental autoimmune thyroiditis. Clin Immunol Immunopathol 54:53–63
Green LM, LaBue M, Lazarus JP, Colburn KK (1995) Characterization of autoimmune thyroiditis in MRL-lpr/lpr mice. Lupus 4:187–196
Green LM, LaBue M, Lazarus JP, Jennings JC (1996) Reduced cell-cell communication in experimentally induced autoimmune thyroid disease. Endocrinology 137:2823–2832
Hassman RA, Dieguez C, Rennie DP, Weetman AP, Hall R, McGregor AM (1985) The influence of cyclosporin A on the induction of experimental autoimmune thyroid disease in the PVG/c rat. Clin Exp Immunol 59:10–16
Iddah MA, Macharia BN (2013) Autoimmune thyroid disorders. ISRN Endocrinol 2013. Article ID 509764, 9 pp. doi:10.1155/2013/509764
McGregor AM, Rennie PD, Weetman AP, Hassman RA, Foord SM, Dieguez C, Hall R (1983) The influence of cyclosporin A on experimental autoimmune thyroid disease in the rat. Life Sci 32:97–108
Luo Y, Kawashima A, Ishido Y, Yoshihara A, Oda K, Hiroi N, Ito T, Ishii N, Suzuki K (2014) Iodine excess as an environmental risk factor for autoimmune thyroid disease. Int J Mol Sci 15:12895–12912
Mori K, Yoshida K, Ishi K, Moroshi K, Nakagawa Y, Hoshikawa S, Ozaki H, Takahashi Y, Ito S (2011) Experimental autoimmune thyroiditis in human parvovirus B10 transgenic mice. Autoimmunity 44:483–489
Penhale WJ, Farmer A, Irvine WJ (1975) Thyroiditis in T cell-depleted rats: influence of strain, radiation dose, adjuvants and antilymphocyte serum. Clin Exp Immunol 21:362–375
Salamero J, Remy JJ, Michel-Béchet M, Chareire J (1987) Experimental autoimmune thyroiditis induced by a 5–10 kDa tryptic fragment from porcine thyroglobulin. Eur J Immunol 17:843–848
Tamura K, Woo J, Murase N, Nalesnik M, Thomson AW (1993) Inhibitory effect of FK 506 on autoimmune thyroid disease in the PVG rat. Ann N Y Acad Sci 696:257–262
Vladutiu AO (1983) Effect of cyclosporine on experimental autoimmune thyroiditis in mice. Transplantation 35:518–520
Vladutiu AO, Rose NR (1971) Autoimmune murine thyroiditis relation to histocompatibility (H-2) type. Science 174:1137–1139
Wang SH, Fan Y, Baker JR Jr (2014) Overexpression of BID in thyroids of transgenic mice increases sensitivity to iodine-induce autoimmune thyroiditis. J Transl Med 12:180

Coxsackievirus B3-Induced Myocarditis

Beisel KW, Srinivasappa J, Prabhakar BS (1991) Identification of a putative shared epitope between Coxsackie virus B4 and alpha cardiac myosin heavy chain. Clin Exp Immunol 86:49–55
Chai D, Yue Y, Xu Y, Dong C, Xiong S (2014) Mucosal co-immunisation with AIM2 enhances protective SIgA response and increases prophylactic efficacy of chitosan-DNA vaccine against coxsackievirus B3-induced myocarditis. Hum Vaccin Ther 10:1284–1294
Estrin M, Huber SA (1987) Coxsackie virus B3-induced myocarditis. Autoimmunity is $L3T4^+$ T helper cell and IL-2 independent in Balb/c mice. Am J Pathol 127:337–341
Estrin M, Smith C, Huber S (1986) Coxsackie virus B-3 myocarditis. T-cell autoimmunity to heart antigens is resistant to cyclosporin-A treatment. Am J Pathol 125:244–251
Estrin M, Herzum M, Buie C, Huber SA (1987) Immunosuppressives in murine myocarditis. Eur Heart J 8(Suppl J):259–262
Fairweather D, Frisancho-Kiss S, Njoku DB, Nyland JF, Kaya Z, Yusung SA, Davis SE, Frisancho JA, Barrett MA, Rose NR (2006) Complement receptor 1 and 2 deficiency increases Coxsackievirus B3-induced myocarditis, dilated cardiomyopathy and heart failure by increasing macrophages, IL-1b, and immune complex deposition in the heart. J Immunol 176:3516–3524
Gauntt CJ, Higdon HL, Arizpe HM, Tamayo MR, Crawley R, Henkel RD, Pereira MEA, Tracy SM, Cunningham MW (1993) Epitopes shared between Coxsackievirus B3 (CVB3) and normal heart tissue contribute to CVB3-induced murine myocarditis. Clin Immunol Immunopathol 68:129–134
He J, Yue Y, Dong C, Xiong S (2013) MiR-21 confers resistance against CVB3-induced myocarditis by inhibiting PDCD4-mediated apoptosis. Clin Invest Med 36:E103–E111
Huber SA, Lodge PA (1984) Coxsackie virus B-3 myocarditis in Balb/c mice. Evidence for autoimmunity to myocyte antigens. Am J Pathol 116:21–29
Huber SA, Lodge PA (1986) Coxsackie virus B-3 myocarditis. Identification of different pathogenic mechanisms in DBA/2 and Balb/c mice. Am J Pathol 122:284–291
Lane JR, Neumann DA, Lafond-Walker A, Herskowitz A, Rose NR (1991) LPS promotes CB3-induced myocarditis in B10. A mice. Cell Immunol 136:219–233
Lv K, Xu W, Wang C, Niki T, Hirashima M, Xiong S (2011) Galectin-9 administration ameliorates CVB3 induced myocarditis by promoting the proliferation of regulatory T cells and alternatively activated Th2 cells. Clin Immunol 140:92–101
Monrad ES, Matsumori A, Murphy JC, Fox JG, Crumpacker CS, Abelmann WH (1986) Therapy with cyclosporine in experimental murine myocarditis with encephalomyocarditis virus. Circulation 7:1058–1064

O'Connell JB, Reap EA, Robinson JA (1986) The effects of cyclosporine on acute murine Coxsackie B-3 myocarditis. Circulation 73:353–359

Onyimba JA, Coronado MJ, Garton AE, Kim JB, Bucek A, Bedja D, Gabrielson KL, Guilarte TR, Fairweather D (2011) The innate immune response to coxsackievirus B3 predicts progression to cardiovascular disease and heart failure in male mice. Biol Sex Differ 2011 Feb 21;2:2. doi: 10.1186/2042-6410-2-2

Park J-H, Kim D-S, Cho Y-J, Kim Y-J, Jeong S-Y, Lee S-M, Cho S-J, Yun C-W, Jo I, Nam J-H (2009) Attenuation of coxsackievirus B3 by VP2 mutation and its application as a vaccine against virus-induced myocarditis and pancreatitis. Vaccine 27:1974–1983

Rose NR, Herskowitz A, Neumann DA (1993) Autoimmunity in myocarditis: models and mechanisms. Clin Immunol Immunopathol 68:95–99

Xie Y, Chen R, Zhang X, Yu Y, Yang Y, Zou Y, Ge J, Chen H (2012) Blockade of interleukin-17A protects against Coxsackievirus B3-induced myocarditis by increasing COX-2/PGE2 production in the heart. FEMS Immunol Med Microbiol 64:343–351

Yu Z, Huang Z, Shao C, Huang Y, Zhang F, Yang J, Deng L, Zeng Z, Deng Q, Zeng W (2011) Oral administration of interferon-a-2b-transformed *Bifidobacterium longum* protects BALB/c mice against Coxsackievirus B3-induced myocarditis. Virol J 8:525

Xu W, Shen Y, Jiang Z, Wang Y, Chu Y, Xiong S (2004) Intranasal delivery of chitosan-DNA vaccine generates mucosal SIgA and anti-CVB3 protection. Vaccine 22:3603–3612

Yue Y, Gui J, Xu W, Xiong S (2011) Gene therapy with CCL2 (MCP-1) mutant protects CVB3-induced myocarditis by compromising Th1 polarization. Mol Immunol 48:706–713

Yue Y, Xu W, Hu L, Jiang Z, Xiong S (2009) Enhanced resistance to coxsackievirus B3-induced myocarditis by intranasal co-immunization of lymphotactin gene encapsulated in chitosan particle. Vaccine 386:438–447

Yue-Chun TZ, Na-Dan Z, Li-Sha G, Qin L, Xue-Qiang G, Jia-Feng L (2012) Comparison of effects of Ivabradine versus Carvedilol in murine model with the Coxsackie virus B3-induced viral myocarditis. PLoS One 7: e39394

Porcine Cardiac Myosin-Induced Autoimmune Myocarditis in Rats

Cammock CE, Halnon NJ, Skoczylas J, Blanchard J, Bohm R, Miller CJ, Lai C, Krogstad PA (2013) Myocarditis, disseminated infection, and early viral persistence following experimental coxsackievirus B infection of cynomolgus monkeys. PLoS One 8: e74569

Dimitrijevic M, Milenkovic M, Milosavljevic P, Stojic-Vukanic Z, Colic M, Bartlett R (1998) Beneficial effects of leflunomide on cardiac myosin-induced experimental autoimmune myocarditis in rats. Int J Immunother 14:9–21

Gong X, Han B, Zou Y, Wang J, Yang W (2014) Attenuation of experimental autoimmune myocarditis by si-RNA mediated CD40 silencing. Int Heart J 55:539–545

Hirano E, Shimada K, Komiyama T, Fulita M, Kishimoto C (2013) Erythromycin treatment suppresses myocardial injury in autoimmune myocarditis in rats via suppression of superoxide production. Int J Cardiol 167:2228–2233

Hoetzenecker K, Zimmermann M, Hoetzenecker W, Schwieger T, Kollmann D, Mildnerr M, Hegedus B, Mitterbauer A, Hacker S, Birner P, Gabriel C, Gyongyosi M, Blyszczuk P, Eriksson U, Ankersmit HJ (2013) Mononuclear cell secretome protects from experimental autoimmune myocarditis. Eur Heart J. doi: 10.1093/eurheartj/ehs459

Inomata T, Hanawa H, Miyanishi T, Yajima E, Nakayama S, Maita T, Kodama M, Izumi T, Shibata A, Abo T (1995) Localization of porcine cardiac myosin epitopes that induce experimental autoimmune myocarditis. Circ Res 76:726–733

Kodama M, Zhang S, Hanawa H, Saeki M, Inomata T, Suzuki K, Koyama S, Shibata A (1995) Effects of 15-deoxyspergualin on experimental autoimmune giant cell myocarditis of the rat. Circulation 91:1116–1122

Koyama S, Kodama M, Izumi T, Shibata A (1995) Experimental rat model representing both acute and chronic failure related to autoimmune myocarditis. Cardiovasc Drugs Ther 9:701–707

Milenkovic M, Arsenovic-Ranan N, Vucicevic D, Bufan B, Stojic-Vukanic Z (2007) Fusidin ameliorates experimental autoimmune myocarditis in rats by inhibiting TNF-α production. Pharmazie 62:445–448

Murakami U, Uchida K, Hiratsuka T (1976) Cardiac myosin from pig heart ventricle: purification and enzymatic properties. J Biochem 80:611

Myers JM, Cunningham MW, Fairweather D, Huber SA (2013) Autoimmune myocarditis, valvulitis and cardiomyopathy. Curr Protoc Immunol. doi: 10.1002/0471142735.im1514s101

Neu N, Ploier B (1991) Experimentally induced autoimmune myocarditis: production of heart myosin-specific antibodies. Autoimmunity 8:317–322

Neu N, Ploier B, Ofner C (1990) Cardiac myosin-induced myocarditis. Heart autoantibodies are not involved in the induction of the disease. J Immunol 145:4094–4100

Neu N, Klieber R, Frühwirth M, Berger P (1991) Cardiac myosin-induced myocarditis as a model of postinfectious autoimmunity. Eur Heart J Suppl D:117–120

Oh H, Ahn M, Matsumoto Y, Shiu T (2013) Alternatively activated M2 macrophages increase in early stages of experimental autoimmune myocarditis in Lewis rats. Korean J Vet Res 53:225–230

Pando R, Barshack I, Raz A, Luboshits G, Haklai R, Maysel-Auslender S, Kloog Y, Keren G, George J

(2010) The Ras antagonist farnesylthiosalicylic acid ameliorates experimental myocarditis in the rat. Cardiovasc Pathol 19:94–101

Penninger JM, Neu N, Timms E, Wallace VA, Koh DR, Kishihara K, Pummerer C, Mak TW (1993) Induction of experimental autoimmune myocarditis in mice lacking CD3 or CD8 molecules. J Exp Med 178:1837–1842

Pummerer C, Berger P, Frühwirth M, Ofner C, Neu N (1991) Cellular infiltrate, major histocompatibility antigen expression and immunopathogenic mechanisms in cardiac myosin-induced myocarditis. Lab Invest 65:538

Sukumaran V, Veeraveedu PT, Gurusamy N, Yamaguchi K, Lakshmanan AP, Ma M, Suzuki K, Kodoma M, Watanabe K (2011) Cardioprotective effects of Telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/Mas receptor axis. Int J Biol Sci 7:1077–1092

Suzuki J-I, Ogawa M, Muto S, Itai A, Isobe M (2008) A specific inhibitor of plasminogen activator inhibitor-1 suppresses rat autoimmune myocarditis. Expert Opin Ther Targets 12:1313–1320

Suzuki K (1995) A histological study on experimental autoimmune myocarditis with special reference to initiation of the disease and cardiac dendritic cells. Virchows Arch 426:493–500

Veia A, Cavallino C, Bacchini S, Pastore F, Lupi A, Rognoni A, Rametta F, Bongo AS (2014) Idiopathic giant cell myocarditis: state of the art. World J Cardiovasc Dis 4:316–324

Wahed MII, Watanabe K, Ma M, Yamaguchi K, Takahashi T, Tachikawa H, Kodame M, Aizawa Y (2005) Effects of eplerenone, a selective aldosterone blocker, on the progression of left ventricular dysfunction and remodeling in rats with dilated cardiomyopathy. Pharmacology 73:81–88

Experimental Allergic Encephalomyelitis

Alvord EC (1984) The challenge: how good a model of MS is EAE today? In: Alvord EC, Kies MW, Suckling AJ (eds) Experimental allergic encephalomyelitis: a useful model for multiple sclerosis. Alan R Liss, New York, pp 3–5

Arnon R (1981) Experimental allergic encephalomyelitis – susceptibility and suppression. Immunol Rev 55:5–30

Baker D, O'Neill JK, Gschmeissner SE, Wilcox CE, Butter C, Turk JL (1990) Induction of chronic relapsing experimental allergic encephalomyelitis in Biozzi mice. J Neuroimmunol 28:261–270

Baker D, O'Neill JK, Turk JL (1991) Cytokines in the nervous system of mice during chronic relapsing experimental allergic encephalomyelitis. Cell Immunol 134:505–510

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman HW, Layward L (2000) Cannabinoids control spasticity and tremor in a multiple sclerosis model. Nature 202:84–87

Ben-Nun A, Cohen IR (1982) Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. J Immunol 129:303–308

Bolton C, Borel JF, Cuzner ML, Davison AN, Turner AM (1982) Immunosuppression by cyclosporin A of experimental allergic encephalomyelitis. J Neurol Sci 56:147–153

Carlson RP, Baeder WL, Caccese RG, Warner LM, Sehgal SN (1992) Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. Ann N Y Acad Sci 685:86–113

Carlson RP, Hartman DA, Tomchek LA, Walter TL, Lugay JR, Calhoun W, Sehgal SN, Chang JY (1993) Rapamycin, a potential disease-modifying antiarthritic drug. J Pharmacol Exp Ther 266:1125–1138

Chabannes D, Ryffel B, Borel JF (1992) SRI 62–834, a cyclic ether analogue of the phospholipid ET-18-OCH_3, displays long-lasting beneficial effects in chronic relapsing encephalomyelitis in the Lewis rat. Comparison with cyclosporin and (Val^2)-dihydrocyclosporin effects in clinical, functional and histological studies. J Autoimmun 5:199–211

Constantinescu CS, Farooqi N, O'Brien K, Gran B (2011) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis. Br J Pharmacol 164:1079–1106

Croxford AL, Kurschus FC, Waisman A (2011) Mouse model for multiple sclerosis: historical facts and future implications. Biochim Biophys Acta 1812:177–183

Dasgupta S, Mazumder B, Ramani YR, Bhattacharyya SP, Das MK (2011) Evaluation of the role of erythropoietin and methotrexate in multiple sclerosis. Indian J Pharm 43:512–515

Deng C, Minguela A, Hussain RZ, Lovett-Racke AE, Radu C, Ward ES, Racke MK (2002) Expression of the tyrosine phosphatase Src homology 2 domain-containing protein tyrosine phosphatase 1 determines T cell activation threshold and severity of experimental autoimmune encephalomyelitis. J Immunol 168:4511–4518

Denic A, Johnson AJ, Bieber AJ, Warrington AE, Rodriguez M, Pirko I (2011) The relevance of animal models in multiple sclerosis research. Pathophysiology 18:21–29

Diab A, Hussain RZ, Lovett-Racke AE, Chavis JA, Drew PD, Racke MK (2004) Ligands for the peroxisome proliferator-activated receptor-γ and the retinoid X receptor exert additive anti-inflammatory effects on experimental autoimmune encephalomyelitis. J Neuroimmunol 148:116–126

Duckers HJ, Muller HJ, Verhaagen J, Nicolay K, Gispen WH (1997) Longitudinal in vivo magnetic resonance

imaging studies in experimental allergic encephalomyelitis : effect of a neurotropic treatment on cortical lesion development. Neuroscience 77:1163–1173

Feurer C, Chow LH, Borel JF (1988) Preventive and therapeutic effects of cyclosporin and valine^2-dihydrocyclosporin in chronic relapsing experimental allergic encephalomyelitis in the Lewis rat. Immunology 63:219–223

Gautam AM, Pearson CI, Smilek DE, Steinman L, McDevitt HO (1992) A polyalanine peptide with only five native basic protein residues induces autoimmune encephalomyelitis. J Exp Med 176:605–609

Genain CP, Hauser SL (1997) Creation of a model for multiple sclerosis in *Callithrix jacchus* marmosets. J Mol Med 75:187–197

Gilgun-Sherki Y, Panet H, Melamed E, Offen D (2003) Riluzole suppresses experimental autoimmune encephalomyelitis: implications for the treatment of multiple sclerosis. Brain Res 989:196–204

Glabinski AR, Tani M, Strieter RM, Tuohy VK, Ransohoff RM (1997) Synchronous synthesis of α- and β-chemokines by cells of diverse lineage in the central nervous system of mice with relapses of chronic experimental autoimmune encephalomyelitis. Am J Pathol 150:617–630

Hartung HP, Schäfer B, Fierz W, Heininger K, Toyka KV (1987) Cyclosporin A prevents P2 T cell line-mediated experimental autoimmune neuritis (AT-EAN) in rat. Neurosci Lett 83:195–200

Heremans H, Dillen C, Groenen M, Martens E, Billiau A (1996) Chronic relapsing experimental autoimmune encephalomyelitis in mice: enhancement by monoclonal antibodies against interferon-gamma. Eur J Immunol 26:2393–2398

Hinrichs DJ, Wegmann KW, Peters BA (1983) The influence of cyclosporin A on the development of actively induced and passively transferred experimental allergic encephalomyelitis. Cell Immunol 77:202–209

King RHM, Craggs RI, Gross MLP, Tompkins C, Thomas PK (1983) Suppression of experimental allergic neuritis by cyclosporin A. Acta Neuropathol (Berl) 59:262–268

Kojima K, Berger T, Lassmann H, Hinze-Selch D, Zhang Y, Gehrmann J, Reske K, Wekerle H, Linington C (1994) Experimental autoimmune panencephalitis and uveoretinitis transferred to the Lewis rat by T lymphocytes specific for the S100 β molecule, a calcium binding protein of astroglia. J Exp Med 180:817–829

Levine S, Sowinski R (1977) Suppression of the hyperacute form of experimental allergic encephalomyelitis by drugs. Arch Int Pharmacodyn Ther 230:309–318

Liblau R, Steinman L, Brocke S (1997) Experimental autoimmune encephalomyelitis in IL-4 deficient mice. Int Immunol 9:799–803

Maron R, Aj S, Hoffmann E, Komagata Y, Weiner HL (2002) Oral tolerance to copolymer 1 in myelin basic protein (MBP) TCR transgenic mice: cross-reactivity with MBP-specific TCR and differential induction of anti-inflammatory cytokines. Int Immunol 14:131–138

Martin D, Near SL (1995) Protective effect of the interleukin-1 antagonist (IL-1ra) on experimental allergic encephalomyelitis in rats. J Neuroimmunol 61:241–245

Massacesi L, Genain CP, Lee-Parritz D, Letvin NL, Canfield D, Hauser SL (1995) Active and passively induced experimental autoimmune encephalomyelitis in common marmosets: a new model of multiple sclerosis. Ann Neurol 37:519–530

Matejuk A, Hopke C, Dwyer J, Subramanian S, Jones RE, Bourdette DN, Vandenbark AA, Offner H (2003) CNS gene expression pattern associated with spontaneous experimental autoimmune encephalomyelitis. J Neurosci Res 73:667–678

McCombe PA, van der Kreek SA, Pender MP (1990) The effects of prophylactic cyclosporin A on experimental allergic neuritis (EAN) in the Lewis rat. Induction of relapsing EAN using low dose of cyclosporin A. J Neuroimmunol 28:131–140

McFarlin DF, Blank SE, Kibler RF, McKneally S, Shapira R (1973) Experimental allergic encephalomyelitis in the rat: response to encephalitogenic proteins and peptides. Science 179:478–483

Mix E, Correale J, Olsson T, Solders G, Link H (1992) Effect of stilbene-type anion channel blockers on the immune response during experimental allergic neuritis. Immunopharmacol Immuntoxicol 14:579–609

Mondal S, Pahan K (2015) Cinnamon ameliorate experimental allergic encephalomyelitis in mice via regulatory T cells: implications for multiple sclerosis therapy. PLoS One 10:e0116566

Nakayasu H, Ota K, Tanaka H, Irie H, Takahashi H (1990) Suppression of actively induced and passively transferred experimental allergic neuritis by cyclosporin A. J Neuroimmunol 26:219–227

Pachner AR (2011) Experimental models of multiple sclerosis. Curr Opin Neurol 24:291–299

Paris D, Beaulieu-Abdelahad D, Mullan M, Ait-Ghezala G, Mathura V, Bachmeier C, Crawford F, Mullan MJ (2013) Amelioration of experimental autoimmune encephalomyelitis by anatabine. PLoS One 8: e55392

Pearson CI, Smilek DE, Danska JS, McDevitt HO (1997) Induction of a heterogeneous TCR repertoire in (PL/JXSJL/J) F2 mice by myelin basic protein peptide Ac1–11 and its analog Ac1–11[4A]. Mol Immunol 14:781–792

Pollak Y, Ovadia H, Orion E, Yimiya R (2003) The EAE-associated behavioral syndrome: II. Modulation by anti-inflammatory treatments. J Neuroimmunol 137:100–108

Polman CH, Matthaei I, de Groot CJA, Koetsier JC, Sminia T, Dijkstra CD (1988) Low-dose cyclosporin A induces relapsing remitting experimental allergic encephalomyelitis in the Lewis rat. J Neuroimmunol 17:209–216

Ratts RB, Arredono LR, Bittner P, Perrin PJ, Lovett-Racke AE, Racke MK (1999) The role of CTLA-4 in tolerance induction and T cell differentiation in experimental autoimmune encephalomyelitis.: i.p. antigen administration. Int Immunol 11:1881–1888

Rivers TM, Sprunt DH, Berry GP (1933) Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. J Exp Med 58:39–53

Rosenthale ME, Datko LJ, Kassarich J, Schneider F (1969) Chemotherapy of experimental allergic encephalomyelitis (EAE). Arch Int Pharmacodyn Ther 179:251–275

Rott O, Cash E, Fleischer B (1993) Phosphodiesterase inhibitor pentoxifylline, a selective suppressor of T helper type 1-but not type 2-associated lymphokine production, prevents induction of experimental autoimmune encephalomyelitis in Lewis rats. Eur J Immunol 23:1745–1751

Schuller-Levis GB, Kozlowski PB, Wisniewski HM (1986) Cyclosporin A treatment of an induced attack in a chronic relapsing model of experimental allergic encephalomyelitis. Clin Immunol Immunopathol 40:244–252

Singhal M, Srivastava P (2012) Experimental autoimmune encephalomyelitis model for discovery of new therapy for multiple sclerosis. Glob J Pharmacol 6:208–215

't Hart BA, Bauer J, Muller HJ, Melchers B, Nicolay K, Brok H, Bontrop RE, Lassmann H, Massacesi L (1998) Histopathological characterization of magnetic resonance imaging-detectable white matter lesions in a primate model of multiple sclerosis. A correlative study in the experimental autoimmune encephalomyelítis model in common marmosets (*Callithrix jacchus*). Am J Pathol 153:649–663

't Hart BA, Vogel J, Bauer J, Brok HPM, Blezer E (2004) Non-invasive measurement of brain damage in a primate model of multiple sclerosis. Trends Mol Med 10:85–91

Tian DH, Perera CJ, Moalem-Taylor G (2013) Neuropathic pain in animal models of nervous system autoimmune diseases. Mediators Inflamm 2013:298326. doi:10.1155/2013/298326

Waksman BH, Adams RD (1955) Allergic neuritis: an experimental disease of rabbits induced by the injection of peripheral nervous tissue and adjuvants. J Exp Med 102:213–234

Waksman BH, Adams RD (1956) A comparative study of experimental allergic neuritis in rabbit, guinea-pig and mouse. J Neuropathol Exp Neurol 15:293–374

Warford J, Robertson GS (2011) New methods for multiple sclerosis drug discovery. Expert Opin Drug Discov 7:689–699

Acute Graft Versus Host Disease (GVHD) in Rats

Bartlett RR, Dimitrijevic M, Mattar T, Zielinski T, Germann T, Rüde E, Thoenes GH, Küchle CCA, Schorlemmer HU, Bremer E, Finnegan A, Schleyerbach R (1991) Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of auto-immune disorders and reactions leading to transplantation rejection. Agents Actions 32:11–21

Bartlett RR, Anagnostopulos H, Zielinski T, Mattar T, Schleyerbach R (1993) Effects of leflunomide on immune responses and models of inflammation. Springer Semin Immunopathol 14:381–394

Caballero-Velázquez T, Sánchez-Abarca LI, Gutierrez-Cosio S, Blanco B, Calderon C, Herrero C, Carrancio S, Serrano C, del Cañizo C, San Miguel JF, Pérez-Simón JA (2012) The novel combination of sirolimus and bortezomib prevents graft-versus-host disease but maintains the graft-versus-leukemia effect after allogeneic transplantation. Haematologica 97:1329–1337

Deol A, Ratanatharathorn V, Uberti JP (2011) Pathophysiology, prevention, and treatment of acute graft-versus-host disease. Transpl Res Risk Manag 3:31–44

Ford WL, Burr W, Simonsen G (1970) A lymph node weight assay for the graft-versus-host activity of rat lymphoid cells. Transplantation 10:258

Gelpi C, Martinez MA, Vidal S, Targoff IN, Rodriguez-Sanchez JL (1994) Antibodies to a transfer RNA-associated protein in a murine model of chronic graft versus host disease. J Immunol 152:1989–1999

Jones KR, Kang EM (2015) Graft versus host disease: new insights into A_{2A} receptor agonist therapy. Comp Struct Biotechnol J 13:101–105

Kim HM, Han SB, Hong DH, Yoo BS, Oh GT (1977) Limitation of Hu-PBL-scid mouse model in direct application to immunotoxicity assessment. J Pharmacol Toxicol Methods 37:83–89

Küchle CCA, Thoenes GH, Langer KH, Schorlemmer HU, Bartlett RR, Schleyerbach R (1991) Prevention of kidney and skin graft rejection in rats by leflunomide, a new immunomodulating agent. Transplant Proc 23:1083–1086

Leventhal J, Huang Y, Xu H, Goode I, Ildstad ST (2012) Novel regulatory therapies for prevention of Graft-versus-host disease. BMC Med 10:48

Mosier DE, Gulizia RJ, Baird SM, Wilson DB (1988) Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature 335:256–259

Mrowka C, Thoenes GH, Langer KH, Bartlett RR (1994) Prevention of acute graft versus host disease (GVHD) in rats by the immunomodulating drug leflunomide. Ann Hematol 68:195–199

Murase N, Demetris AJ, Woo J, Tanabe M, Furuya T, Todo S, Starzl TE (1993) Graft-versus-host disease after Brown Norway-to-Lewis and Lewis-to-Brown Norway rat intestinal transplantation under FK 506. Transplantation 55:1–7

Punj S, Koppaparu P, Jang HS, Phillips JL, Pennington J, Rohlman D, O'Donnell E, Iverson PL, Kolluri SK, Kerkvliet NI (2014) Benzimidazoisoquinolines: a new class of rapidly metabolized aryl hydrocarbon receptor

(AhR) ligands that induce AhR-dependent tregs and prevent murine graft-versus-host disease. PLoS One. doi: 10.1371/journal.pone.0088726
Renkonen R, Häyry P (1984) Bone marrow transplantation in the rat. I. Histologic correlation and quantification of cellular infiltrates in acute graft-versus-host disease. Am J Pathol 117:462–470
Schorlemmer HU, Seiler FR, Bartlett RR (1993) Prolongation of allogenetic transplanted skin grafts and induction of tolerance by leflunomide, a new immunosuppressive isoxazol derivative. Transplant Proc 25:763–767
Schorlemmer HU, Kurrle R, Bartlett R (1997) The new immunosuppressants, the malononitrilamides MNA 279 and MNA 715, inhibit various graft-vs.-host diseases (GvHD) in rodents. Drugs Exp Clin Res 23:167–173
Schorlemmer HU, Kurrle R, Schleyerbach R (1998) A77–1726, leflunomide's active metabolite, inhibits in vivo lymphoproliferation in the popliteal lymph node assay. Int J Immunother 14:205–211
Shaffer D, Muanza T, Blakely ML, Simpson MA, Monaco AP (1993) Prevention of graft-versus-host-disease by RS-61443 in two different rodent models. Transplantation 55:221–223
Strober S (2014) Path to clinical transplantation tolerance and prevention of graft versus host disease. Immunol Res 58:240–248
Thoenes GH, Sitter T, Langer KH, Bartlett RR, Schleyerbach R (1989) Leflunomide (HWA 486) inhibits experimental autoimmune tubulointerstitial nephritis in rats. Int J Immunopharmacol 11:921–929
Wakely E, Oberholser JH, Corry RJ (1990) Elimination of acute GVHD and prolongation of rat pancreas allograft survival with DST, cyclosporine, and spleen transplantation. Transplantation 49:241–245
Xia X, Liang C, Liu H, Xue F, Hu Q, Chen W, Ma T, Zhang Y, Bai X, Liang T (2013) Effects of trichostatin A in a rat model of acute graft-versus-host disease after liver transplantation. Transplantation 96:25–33
Xu G, Wang L, Chen W, Xue F, Bai X, Liang L, Shen X, Zhang M, Xia D, Liang T (2010) Rapamycin and Tacrolimus differentially modulate acute graft-versus-host disease in rats after liver transplantation. Liver Transpl 16:357–363
Zeiser R (2014) Animal models of graft-versus-host disease. Hematol Educ 8:359–366

Influence on SLE-Like Disorder in MRL/lpr Mice

Avci P, Sadasivam M, Gupta A, De Melo WCMA, Huang Y-Y, Yin R, Rakkiyappan C, Kumar R, Otufowora A, Nyame T, Hamblin MR (2013) Animal models of skin disease for drug discovery. Expert Opin Drug Discov 8:331–355
Bartlett RR, Popovic S, Raiss RX (1988) Development of autoimmunity in MRL/lpr mice and the effect of drugs on this murine disease. Scand J Rheumatol Suppl 75:290–299
Bartlett RR, Mattar T, Weithmann U, Anagnostopulos H, Popovic S, Schleyerbach R (1989) Leflunomide (HWA 486): a novel immunorestoring drug. In: Lewis AJ, Doherty NS, Ackerman NR (eds) Therapeutic approaches to inflammatory diseases. Elsevier Science, New York, pp 215–228
Bender AT, Wu Y, Cao Q, Ding Y, Oestreicher J, Genest M, Akare S, Ishizaka ST, Mackey MF (2014) Assessment of the translational value of mouse lupus models using clinically relevant biomarkers. Transl Res 163:515–532
Boissier MC, Texier B, Carlioz A, Fournier C (1989) Polyarthritis in MRL-1pr/1pr mice: mouse type II collagen is antigenic, but not arthritogenic. Autoimmunity 4:31–41
Bundick RV, Eady RP (1992) The effects of CP 17193, an immunosuppressive pyrazoloquinoline, on the development of spontaneous lupus disease in NZBW F_1 hybrid mice. Clin Exp Immunol 89:179–184
Carlson RP, Baeder WL, Caccese RG, Warner LM, Sehgal SN (1992) Effects of orally administered rapamycin in animal models of arthritis and other autoimmune diseases. Ann N Y Acad Sci 685:86–113
Chan CC, Gery I, Kohn LD, Nussenblatt RB, Mozes E, Singer SD (1995) Periocular inflammation in mice with experimental systemic lupus erythematodes. A new experimental blepharitis and its modulation. J Immunol 154:4830–4835
Furukawa F, Kanauchi H, Wakita H, Tokura Y, Tachibana T, Horiguchi Y, Imamura S, Ozaki S, Takigawa M (1996) Spontaneous autoimmune skin lesions of MRL/n mice: autoimmune disease-prone genetic background in relation to Fas-defect MRL/lpr mice. J Invest Dermatol 107:95–100
Gleichmann E, van Elven EH, van der Veen JPW (1982) A systemic lupus erythematodes (SLE)-like disease in mice induced by abnormal T- and B-cell cooperation. Preferential formation of antibodies characteristic of SEL. Eur J Immunol 12:152
Gunn HC, Hiestand PC (1988) Cyclosporine A and cyclosporine G enhance IgG rheumatoid factor production in MRL/Ipr mice. Transplant Proc 20(Suppl 4):238–242
Holmdahl R, Tarkowski A, Jonsson R (1991) Involvement of macrophages and dendritic cells in synovial inflammation of collagen induced arthritis in DBA/1 mice and spontaneous arthritis in MRL/Lpr mice. Autoimmunity 8:271–280
Jeltsch-David H, Muller S (2014) Neuropsychiatric systemic lupus erythematosus and cognitive dysfunction: the MRL/lpr mouse strain as a model. Autoimmun Rev 13:963–973
Kanno H, Nose M, Itoh J, Taniguchi Y, Kyogoku M (1992) Spontaneous development of pancreatitis in the MRL/Mp strain of mice in autoimmune mechanism. Clin Exp Immunol 89:68–73

Kiberd BA, Stadnyk AW (1995) Established murine lupus nephritis does not respond to exogenous interleukin-1 receptor antagonist: a role for the endogenous molecule? Immunopharmacology 30:131–137

Konya C, Kyttaris VC (2013) T cells as treatment targets in systemic lupus erythematosus. Rheumatol Curr Res 3:120. doi: 10.4172/2161-1149.1000120

Knight JS, Subramanian V, O'Dell AA, Yalavarhi S, Zhao W, Smith CK, Hodgin JB, Thompson PR, Kaplan MJ (2014) Peptidylarginine deaminase inhibition disrupts NET formation and protects against kidney, skin and vascular disease in lupus-prone MRL/lpr mice. Ann Rheum Dis. doi: 10.1136/annrheumdis-2014-205365

Kusakari C, Hozawa K, Koike S, Kyogoku M, Takasaka T (1992) MRL/MP-lrp/Lrp mouse as a model of immune-induced sensorineural hearing loss. Ann Otol Rhinol Laryngol 101:82–86

Kyttaris VC, Kampagianni O, Tsokos GC (2013) Treatment with Anti-Interleukin 23 Antibody Ameliorates Disease in Lupus-Prone Mice. BioMed Res Int 2013. Article ID 861028, 5 pp. doi:10.1155/2013/861028

Marcinko K, Parsons T, Lerch JP, Sled JG, Sakic B (2012) Effects of prolonged treatment with memantine in the MRL model of central nervous system lupus. Clin Exp Neuroimmunol 3:116–128

Markopoulou A, Kyttaris VC (2013) Small molecules in the treatment of systemic lupus erythematosus. Clin Immunol 148:359–368

Richard EM, Thiyagarajan T, Bunni MA, Basher F, Roddy PO, Siskind LJ, Nietart PJ, Nowling TK (2013) Reducing FLI1 levels in the MRL/lpr lupus mouse model impacts T cell function by modulating glycosphingolipid metabolism. PLoS One. doi: 10.1371/kournal.pone.0075175

Rordorf-Adam C, Serban D, Pataki A, Gruninger M (1985) Serum amyloid P component and autoimmune parameters in the assessment of arthritis in MRL/lpr/lpr mice. Clin Exp Immunol 61:509–516

Rottman JB, Willis CR (2010) Mouse models of systemic lupus erythematosus reveal a complex pathogenesis. Vet Pathol 47:664–676

Schorlemmer HU, Dickneite G (1992) Preclinical studies with 15-deoxyspergualin in various animal models for autoimmune diseases. Ann N Y Acad Sci 685:155–174

Schorlemmer HU, Dickneite G, Enßle KH (1995) Immunoregulation of murine SLE-like diseases by interleukin-4-receptor. Lupus 4(Suppl 2):8

Schorlemmer HU, Kurrle R, Bartlett R (1997) The new immunosuppressants, the malononitrilamides MNA 279 and MNA 715, inhibit various graft-vs.-host diseases (GvHD) in rodents. Drugs Exp Clin Res 23:167–173

Shinsuke N, Hiroshi I (2013) Over-expression of Epstein-Barr virus-induced gene 3 protein (EIB3) in MRL/lpr mice suppresses their lupus nephritis by activating regulatory T cells. Autoimmunity 46:446–454

Sullivan DA, Edwards JA (1997) Androgen stimulation of lacrimal gland function in mouse models of Sjögren's syndrome. J Steroid Biochem Mol Biol 60:237–245

Theofilopoulos AN, Dixon FJ (1981) Etiopathogenesis of murine SLE. Immunol Rev 55:179–216

Toda I, Sullivan BD, Rocha EM, Da Silveira LA, Wickham LA, Sullivan DA (1999) Impact of gender on exocrine gland inflammation in mouse models of Sjögren's syndrome. Exp Eye Res 69:355–366

Walker SE, Keisler LW, Caldwell CW, Kier AB, Vom Saal FS (1996) Effects of altered prenatal hormonal environment on expression of autoimmune disease in NZB/NZW mice. Environ Health Perspect 104(Suppl 4):815–821

Williams S, Stafford P, Hoffman SA (2014) Diagnosis and early detection of CNS-SLE in MRL/lpr mice using peptide microarrays. BMC Immunol 15:23

Zoja C, Corna D, Benedetti G, Morigi M, Donadelli R, Guglielmotti A, Pinza M, Bertani T, Remuzzi G (1998) Bindarit retards renal disease and prolongs survival in murine lupus autoimmune disease. Kidney Int 53:726–734

Prevention of Experimentally Induced Myasthenia Gravis in Rats

Arag S, Blalock JE (1994) Use of complementary peptides and their antibodies in B-cell-mediated autoimmune disease: prevention of autoimmune myasthenia gravis with a peptide vaccine. Immunomethods 5:130–135

Damjanovic M, Vidic-Dankovic B, Kosec D, Isakovic K (1993) Thymus changes in experimentally induced myasthenia gravis. Autoimmunity 15:201–207

Drachman DB, Adams RN, McIntosh K, Pestronk A (1985) Treatment of experimental myasthenia gravis with cyclosporin A. Clin Immunol Immunopathol 34:174–188

Harrison C (2012) Neuromuscular disorders: troponin activator improves muscle function. Nat Rev Drug Discov 11:272–273

Itoyama Y, Kira J, Fuji N, Goto I, Yamamoto N (1989) Increases in helper inducer T cells and activated T cells in HTLV-1 associated myelopathy. Ann Neurol 26:257–262

Lennon VA, Lambert EH, Leiby KR, Okarma TB, Talib S (1991) Recombinant human acetylcholine receptor α-subunit induces chronic experimental autoimmune myasthenia gravis. J Immunol 146:2245–2248

McIntosh KR, Drachman DB (1986) Induction of suppressor cells specific for AChR in experimental autoimmune myasthenia gravis. Science 232:401–403

McIntosh KR, Drachman DB (1987) Properties of suppressor cells induced to acetylcholine receptor using cyclosporin A. Ann N Y Acad Sci 505:628–638

Mrowka C, Thoenes GH, Langer KH, Bartlett RR (1994) Prevention of acute graft versus host disease (GVHD)

in rats by the immunomodulating drug leflunomide. Ann Hematol 68:195–199
Norcross NL, Griffith IJ, Lettieri JA (1980) Measurement of acetylcholine receptors and anti-receptor antibodies by ELISA. Muscle Nerve 3:345–349
Oliveira L, Correia A, Costa AC, Guerra-Gomes S, Ferreirinha F, Magalhaes-Cardoso MT, Vilanova M, Correia-de-Sa P (2015) Deficits in endogenous adenosine formation by ecto-5′-nucleotidase/CD73 impair neuromuscular transmission and immune competence in experimental autoimmune myasthenia gravis. Mediat Inflamm 2015. Article ID 460610, 16 pp. doi:10.1155/2015/460610
Oosterhuis H (1981) Observations of the natural history of myasthenia gravis and effect of thymectomy. Ann N Y Acad Sci 377:678–682
Punga AR, Kaminski HJ, Richman DP, Benatar M (2015) How clinical trials of myasthenia gravis can inform pre-clinical drug development. Exp Neurol. doi: 10.1016/j.expneurol.2014.12.022
Russell AJ, Hartman JJ, Hinken AC, Muci AR, Kawas R, Driscoll L, Godinez G, Lee KH, Marquez D, Browne WF 4th, Chen MM, Clarke D, Collibee SE, Garard M, Hansen R, Jia Z, Lu PP, Rodriguez H, Saikali KG, Schaletzky J, Vijayakumar V, Albertus DL, Claflin DR, Morgans DJ, Morgan BP, Malik FI (2012) Activation of fast skeletal muscle troponin as a potential therapeutic approach for treating neuromuscular diseases. Nat Med 18:452–455
Tasgal J, Vaughan-Williams EM (1981) The effect of prolonged propranolol administration on myocardial transmural capillary density in young rabbits. J Physiol 315:353–367
Ulrichs K, Kaitschick J, Bartlett R, Müller-Ruchholtz W (1992) Suppression of natural xenophile antibodies with the novel immunosuppressive drug leflunomide. Transplant Proc 24:718–719
Weible ER (1963) Principles and methods for the morphometric study of the lung and other organs. Lab Invest 12:131–155
Williams JW, Xiao F, Foster P, Clardy C, McChesney L, Sankary H, Chong ASF (1994) Leflunomide in experimental transplantation. Control of rejection and alloantibody production, reversal of acute rejection, and interaction with cyclosporine. Transplantation 57:1223–1231

Glomerulonephritis Induced by Antibasement Membrane Antibody in Rats

Aten J, Bosman CB, de Heer E, Hoedemaeker PJ, Weening JJ (1988) Cyclosporin A induces long-term unresponsiveness in mercuric chloride-induced autoimmune glomerulonephritis. Clin Exp Immunol 73:307–311
Baran D, Vendeville B, Vial MC, Cosson C, Bascou C, Teychenne P, Druet P (1986) Effect of cyclosporin A on mercury-induced autoimmune glomerulonephritis in the Brown Norway rat. Clin Nephrol 25(Suppl 1): S175–S180
Cattran DC (1988) Effect of cyclosporin on active Heymann nephritis. Nephron 48:142–148
D'Souza Z, McAdoo SP, Smith J, Pusey CD, Cook HT, Behmoaras J, Aitman TJ (2013) Experimental crescentic glomerulonephritis: a new bicongenic rat model. Dis Model Mech 6:1477–1486
Fujita M, Ilida H, Asaka M, Izumino K, Takata M, Sasayama S (1991) Effect of the immunosuppressive agent, cyclosporin, on experimental immune complex glomerulonephritis in rats. Nephron 57:210–215
Giménez A, Leyva-Cobian F, Fiero C, Rio M, Bricio T, Mampaso F (1987) Effect of cyclosporin A on autoimmune tubulointerstitial nephritis in the brown Norway rat. Clin Exp Immunol 69:550–556
Grönhagen-Riska C, von Willebrand E, Tikkanen T, Honkanen E, Miettinen A, Holthöfer H, Törnroth T (1990) The effect of cyclosporin A on the interstitial mononuclear cell infiltration and the induction of Heymann's nephritis. Clin Exp Immunol 79:266–272
Heymann W, Hackel DB, Harwood S, Wilson SGF, Hunter JLP (1959) Production of nephrotic syndrome in rats by Freund's adjuvants and rat kidney suspension. Proc Soc Exp Biol Med 100:660–664
Ito M, Yamada H, Okamoto K, Suzuki Y (1983) Crescentic type nephritis induced by anti-glomerular basement membrane (GMB) serum in rats. Jpn J Pharmacol 33:1145–1154
Kokui K, Yoshikawa N, Nakamura H, Itoh H (1992) Cyclosporin reduces proteinuria in rats with aminonucleoside nephrosis. J Pathol 166:297–301
Lan HY, Nikolic-Paterson DJ, Mu W, Vannice JL, Atkins RC (1995) Interleukin-1 receptor antagonist halts the progression of established crescentic glomerulonephritis in the rat. Kidney Int 47:1303–1309
Lillevang ST, Rosenkvist J, Andersen CB, Larsen S, Kemp E, Kristensen T (1992) Single and combined effects of the vitamin D analogue KH1060 and cyclosporin A on mercuric-chloride-induced autoimmune disease in the BN rat. Clin Exp Immunol 88:301–306
Lundstrom M, Orlando RA, Saedi MS, Woodward L, Kurihara H, Farquhar MG (1993) Immunocytochemical and biochemical characterization of the Heymann nephritis antigenic complex in rat L2 yolk sac cells. Am J Pathol 143:1423–1435
Ogawa T, Inazu M, Gotoh K, Hayashi S (1990) Effects of leflunomide on glomerulonephritis induced by antibasement membrane antibody in rats. Agents Actions 31:321–328
Ogawa T, Inazu M, Gotoh K, Inoue T, Hayashi S (1991) Therapeutic effects of leflunomide, a new antirheumatic drug, on glomerulonephritis induced by the

antibasement antibody in rats. Clin Immunol Immunopathol 61:103–118
Reynolds J, Cashman SJ, Evans DJ, Pusey CD (1991) Cyclosporin A in the prevention and treatment of experimental autoimmune glomerulonephritis in the brown Norway rat. Clin Exp Immunol 85:28–32
Schorlemmer HU, Dickneite G (1992) Preclinical studies with 15-deoxyspergualin in various animal models for autoimmune diseases. Ann N Y Acad Sci 685:155–174
Shibata S, Nagasawa T, Takuma T, Naruse T, Miyakawa Y (1966) Isolation and properties of the soluble antigen specific for the production of nephrotoxic glomerulonephritis. I. Immunopathological demonstration of the complete antigenicity of the soluble antigen. Jpn J Exp Med 36:127–143
Shih W, Hines WH, Neilson EG (1988) Effects of cyclosporin A on the development of immune-mediated interstitial nephritis. Kidney Int 33:1113–1118
Smith J, McDaid JP, Bhangal G, Chawanasuntorapoj R, Masuda ES, Cook HT, Pusey CD, Tam FWK (2010) A spleen tyrosine kinase inhibitor reduces the severity of established glomerulonephritis. J Am Soc Nephrol 21:231–236
Suana AJ, Tuffin G, Frey BM, Knudsen L, Muhlfeld C, Rodder S, Marti H-P (2011) Single application of low dose mycophenolate mofetil-OX7- immunoliposomes ameliorates experimental mesangial proliferative glomerulonephritis. J Pharmacol Exp Ther 337:411–422
Takakura K, Mizukami K, Mitori H, Noto T, Tomura Y (2014) Antiproteinuric effect of pirfenidone in a rat model of anti-glomerular basement membrane glomerulonephritis. Eur J Pharmacol 737:106–116
Taylor SRJ, Turner CM, Elliott JI, McDaid J, Hewitt R, Smith J, Pickering MC, Whitehouse DL, Cook HT, Burnstock G, Pusey CD, Unwin RJ, Tam FWK (2009) $P2X_7$ deficiency attenuates renal injury in experimental glomerulonephritis. J Am Soc Nephrol 20:1275–1281
Thoenes GH, Umscheid T, Sitter T, Langer KH (1987) Cyclosporin A inhibits autoimmune experimental tubulointerstitial nephritis. Immunol Lett 15:301–306
Thoenes GH, Sitter T, Langer KH, Bartlett RR, Schleyerbach R (1989) Leflunomide (HWA 486) inhibits experimental autoimmune tubulointerstitial nephritis in rats. Int J Immunopharmacol 11:921–929
Tipping PG, Holdsworth SR (1985) Effect of cyclosporin A on antibody-induced experimental glomerulonephritis. Nephron 40:201–205
Tipping PG, Neale TJ, Holdsworth SR (1985) T lymphocyte participation in antibody-induced experimental glomerulonephritis. Kidney Int 27:530–537
Wilson CB (1981) Nephritogenic antibody mechanisms involving antigens within the glomerulus. Immunol Rev 55:257–297
Wood A, Adu D, Birtwistle RJ, Brewer DB, Michael J (1988) Cyclosporin A and anti-glomerular basement membrane antibody glomerulonephritis in rats. Br J Pathol 69:189–193

Inhibition of Allogenic Transplant Rejection

Adams DH, Tiney NL, Collins JJ, Karnovsky MJ (1992) Experimental graft arteriosclerosis. Transplantation 53:1115–1119
Almond PS, Moss A, Nakhleh R, Melin M, Chen S, Salazar A, Shirabe K, Matas A (1992) Rapamycin in a renal transplant model. Ann N Y Acad Sci 685:121–122
Bartlett RR, Dimitrijevic M, Mattar T, Zielinski T, Germann T, Rüde E, Thoenes GH, Küchle CCA, Schorlemmer HU, Bremer E, Finnegan A, Schleyerbach R (1991) Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of autoimmune disorders and reactions leading to transplantation rejection. Agents Actions 32:11–21
Chesneau M, Michel L, Degauque N, Brouard S (2013) Regulatory B cells and tolerance in transplantation: from animal models to human. Front Immunol 4:1–8
Coupland SE, Klebe S, Karow AC, Krause L, Kruse H, Bartlett RR, Hoffmann F (1994) Leflunomide therapy following penetrating keratoplasty in the rat. Graefe's Arch Clin Exp Ophthalmol 232:622–627
Cramer DV, Chapman FA, Wu GD, Harnaha JB, Qian S, Makowka L (1990) Cardiac transplantation in the rat. Transplantation 50:554–558
Davreux CJ, Chu NH, Waddell TK, Mayer E, Patterson GA (1993) Improved tracheal allograft viability in immunosuppressed rats. Ann Thorac Surg 55:131–134
de Masi R, Alqaisi M, Araneda D, Nifong W, Thomas J, Gross U, Swanson M, Thomas F (1990) Reevaluation of total-lymphoid irradiation and cyclosporine therapy in the Syrian hamster-to-Lewis rat cardiac xenograft model. Transplantation 49:639–641
Engelbrecht G, Kahn D, Duminy F, Hickman R (1992) New rapid technique for renal transplantation in the rat. Microsurgery 13:340–344
Fujino Y, Kawamura T, Hullett DA, Sollinger HW (1994) Evaluation of cyclosporine, mycophenolate mofetil, and brequinar sodium combination therapy on hamster-to-rat cardiac xenotransplantation. Transplantation 57:41–46
Gill P, Jalili R, Ghahary A (2013) Immuno-modulatory role of indoleamine 2,3-dioxygenase in allogeneic islet and skin transplantation. Res Immunol Int J 2013. Article ID 235635. doi:10.5171/2013.235635
Hancock WW, diStefano R, Braun P, Schweizer RT, Tilney NL, Kupiec-Weglinski JW (1990) Cyclosporin and anti-interleukin 2 receptor monoclonal antibody therapy suppress accelerated rejection of rat cardiac allografts through different effector mechanisms. Transplantation 49:416–421
Kahn DR, Forrest DE, Otto DA (1991) Prolonged survival of rat cardiac allografts by donor pretreatment with methotrexate. Transplantation 51:697–700

Katayama Y, Yada I, Namikawa S, Kusagawa M (1991) Immunosuppressive effects of FK 506 in rat lung transplantation. Transplant Proc 23:3300–3301

Kellnar S, Herkomer C, Bae S, Schumacher U (1990) Allogenic transplantation of fetal rat intestine: anastomosis to the normal bowel of the host. J Pediatr Surg 25:415–417

Kim HM, Han SB, Hong DH, Yoo BS, Oh GT (1997) Limitation of Hu-PBL-scid mouse model in direct application to immunotoxicity assessment. J Pharmacol Toxicol Methods 37:83–89

Kirsch AJ, Kirsch SS, Kimura K, LaRosa CA, Jaffe BM (1991) The adaptive ability of transplanted rat small intestine. Surgery 109:779–787

Kobayashi J, Mavroudis C, Crawford SE, Zales VR, Backer CL (1993) A new rat infection-heart transplant model: effect of infection on graft survival studies. J Heart Lung Transplant 12:659–664

Küchle CCA, Thoenes GH, Langer KH, Schorlemmer HU, Bartlett RR, Schleyerbach R (1991) Prevention of kidney and skin graft rejection in rats by leflunomide, a new immunomodulating agent. Transplant Proc 23:1083–1086

Kuroki H, Ishida O, Daisaku H, Fukuhara K, Hatano E, Murakami T, Ikuta Y, Matsumoto AK, Akiyama M (1991) Morphological and immunological analysis of rats with long-term-surviving limb allografts induced by a short course of FK 506 or cyclosporine. Transplant Proc 23:516–520

Langrehr JM, Hoffman RA, Banner B, Stangl MJ, Monyhan H, Le KKW, Schraut WH (1991) Induction of graft-versus-host disease and rejection by sensitized small bowel allografts. Transplantation 52:399–405

Lee S (1967) An improved technique of renal transplantation in the rat. Surgery 61:771

Lee WP, Pan YC, Kesmarky S, Randolph MA, Fiala TS, Amarante MTJ, Weiland AJ, Yaremchuk MJ (1995) Experimental orthotopic transplantation of vascularized skeletal allografts: functional assessment and long-term survival. Plast Reconstr Surg 95:336–353

McManus RP, O'Hair DP, Komorowski R, Scott JP (1993) Immunosuppressant combinations in primate cardiac xenografts. Ann N Y Acad Sci 969:281–284

Mennander A, Tiisala S, Paavonen T, Halttunen J, Häyry P (1991) Chronic rejection of rat aortic allograft. II. Administration of cyclosporin induces accelerated allograft arteriosclerosis. Transpl Int 4:173–179

Muramatsu K, Doi K, Kawai S (1994) The outcome of neurovascularized allogenic muscle transplantation under immunosuppression with cyclosporine. J Reconstr Microsurg 10:77–81

Murase N, Demetris AJ, Woo J, Tanabe M, Furuya T, Todo S, Starzl TE (1993) Graft-versus-host disease after Brown Norway-to-Lewis and Lewis-to-Brown Norway rat intestinal transplantation under FK 506. Transplantation 55:1–7

Nemoto K, Sugawara Y, Mae T, Hayashi M, Abe F, Fujii A, Takeuchi T (1992) Therapeutic activity of deoxyspergualin in comparison with cyclosporin A, and its combined use with cyclosporin A and prednisolone in highly allogeneic skin transplantation in the rat. Agents Actions 36:306–311

Peters DH, Fitton A, Plosker GL, Faulds D (1993) Tacrolimus. A review of its pharmacology, and therapeutic potential in hepatic and renal transplantation. Drug 46:746–794

Sanchez-Fueyo A, Strom TB (2011) Immunologic basis of graft rejection and tolerance following transplantation of liver or other solid organs. Gastroenterology 140:51–64

Schorlemmer HU, Kurrle R (1997) Synergistic activity of malononitrilamides with cyclosporine to control and reverse xenograft rejection. Int J Tissue React 19:149–156

Schorlemmer HU, Seiler FR, Bartlett RR (1993b) Prolongation of allogenetic transplanted skin grafts and induction of tolerance by leflunomide, a new immunosuppressive isoxazol derivative. Transplant Proc 25:763–767

Schroeder MA, DiPersio JF (2011) Mouse models of graft-versus-host disease: advances and limitations. Dis Model Mech 4:318–333

Schuurman HJ, Joergensen J, Kuipers H, Meerloo T, Lardelli P, Hiestand P, White DH, Schreier MH (1994) Vascular transplantation of Syrian hamster heart into Lewis rat: effect of brequinar, cyclosporine, cobra venom factor, and splenectomy. Transplant Proc 26:1217–1219

Shaffer D, Muanza T, Blakely ML, Simpson MA, Monaco AP (1993) Prevention of graft-versus-host-disease by RS-61443 in two different rodent models. Transplantation 55:221–223

Shiraishi M, Csete M, Yasunaga C, McDiarmid SV, Vannice JL, Busuttil RW, Shaked A (1995) The inhibitor cytokine interleukin-1 receptor antagonist synergistically augments cyclosporine immunosuppression in a rat cardiac allograft model. J Surg Res 58:465–470

Steinbrüchel DA, Madsen HH, Nielsen B, Kemp E, Larsen S, Koch C (1991) The effect of combined treatment with total lymphoid irradiation, cyclosporin A, and anti-CD4 monoclonal antibodies in a hamster-to-rat heart transplantation model. Transplant Proc 23:579–580

Svensson G, Holmberg SB, Friman S (1995) Influence of liver transplantation and cyclosporin on bile secretion – an experimental study in the rat. Transpl Int 8:27–34

Thoenes GH, Urban G, Doering I (1974) Kidney transplantation between congenic versus standard inbred strains of rats. I. The significance of H-1 and non-H-I gene differences. Immunogenet 3:239–253

Ulrichs K, Kaitschick J, Bartlett R, Müller-Ruchholtz W (1992) Suppression of natural xenophile antibodies with the novel immunomodulating drug leflunomide. Transplant Proc 24:718–719

van den Bogaerde J, Aspinall R, Wang MW, Cary N, Lim S, Wright L, White D (1991) Induction of long-term survival of hamster heart xenografts in rats. Transplantation 52:15–20

Walpoth BH, Tschopp A, Lazeyras F, Galdikas J, Tschudi J, Altermatt H, Schaffner T, Aue WP, Althaus U (1993) Magnetic resonance spectroscopy for assessing myocardial rejection in the transplanted rat heart. J Heart Lung Transplant 12:271–282

Williams JW, Xiao F, Foster P, Chong A, Sharma S, Bartlett RR, Sankary HN (1993) Immunosuppressive effects of leflunomide in a cardiac allograft model. Transplant Proc 25:745–746

Williams JW, Xiao F, Foster P, Clardy C, McChesney L, Sankary H, Chong ASF (1994) Leflunomide in experimental transplantation. Control of rejection and alloantibody production, reversal of acute rejection, and interaction with cyclosporine. Transplantation 57:1223–1231

Woo J, Valdivia LA, Pan F, Celli S, Fung JJ, Thomson AW (1993) Cytidine potentiates the inhibitory effect of brequinar sodium on xeno-MLR, antibody production, and concordant hamster to rat cardiac xenograft survival. Ann N Y Acad Sci 969:227–234

Xia W, Kirkman RL (1990) Immune function in transplanted small intestine. Transplantation 49:277–280

Xiao F, Chong ASF, Bartlett RR, Williams JW (1994) Leflunomide: a promising immunosuppressant in transplantation. In: Thomson AW, Starzl TE (eds) Immunosuppressive drugs. Edward Arnold, London/Boston/Melbourne, pp 203–212

Yu LT, England J, Sumner A, Larossa D, Hickey WF (1990) Electrophysiologic evaluation of peripheral nerve regeneration through allografts immunosuppressed with cyclosporin. J Reconstr Microsurg 6:317–323

Zhang X, Liu Y, Zhang G, Shi J, Zhang X, Zheng X, Jiang AT, Zhang Z-X, Johnston N, Siu KS, Chen R, Lian D, Koos D, Quan D, Min W-P (2014) Synergic silencing of costimulatory molecules prevents cardiac allograft rejection. J Trans Med 12:142. doi:10.1186/1479-5876-12-142