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Meeting report

Highlights of the joint 14th European Workshop on Inflammation and British Inflammation Research Association Meeting, London, July 1–3, 1992

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Continuing the trend started in Verona in 1991, the 14th European Workshop on Inflammation was jointly organized with another society, this time the British Inflammation Research Association, thereby increasing the programme to three days. The extended format, as in Verona with the Side-Effects of NSAIDs meeting, enabled a larger number of lectures to be presented with a broader thematic coverage than has been usual in the past.

The first day was devoted to neurogenic pain and inflammation and on the succeeding days, cell adhesion molecules in inflammation and future trends in inflammation therapy were the major topics. Morning and afternoon sessions were introduced by invited lecturers and several of these are summarized in this report. The rest of the proceedings contained in this volume are comprised mainly of free communications and posters presented at the meeting.

Neurogenic pain and inflammation

The very close juxtaposition of sensory nerves and effector organs of the immune system ensures that neurohormones inevitably influence immune and inflammatory responses in a variety of ways (E. Weihe, Mainz, Germany). The predominant peptide transmitters at lymph nodes and the thymus are substance P in neurones from the spinal ganglia, vasoactive intestinal peptide (VIP) in neurones from the vagal nerve and neuropeptide Y in neurones of the postganglionic sympathetic nerves. In sensory neurones the predominant neuropeptides are the tachykinins and calcitonin gene related peptide (CGRP), which are detectable in close association with blood vessels, mast cells and macrophages.

Following an inflammatory stimulus, such as the injection of Freund's complete adjuvant into the rat paw, there is a massive depletion of CGRP followed by repletion, as determined by immunochemical staining after 4–6 days. In response to this neurogenic inflammatory mediator release, massive enkephalin release occurs in the spinal cord presumably in order to suppress the painful inflammation in the paw.

One of the most important direct stimulators of pain receptors (nociceptors) during inflammatory responses is bradykinin (BK) (H. P. Rang, London, UK). As shown on rat voltage-clamped sensory neurones, BK stimulates B_2 receptors and initiates an inward Na^+ current in the neurone. This action and the resulting hyperalgesia can be potentiated by prostaglandin E_2 (PGE_2). The generation of the inward current leads to release of CGRP in the spinal cord.

Nociceptive neurones can also be stimulated selectively by capsaicin, an extract of chile peppers which causes neuropeptide release and subsequent depletion. Nerve growth factor (NGF), on the other hand, increases sensitivity of neurones to capsaicin and may do so during inflammation since it is released under the action of cytokines during inflammation and enhances neuropeptide expression and nociception.

There is increasing circumstantial evidence that

neurogenic inflammation is involved in the aetiology of asthma (P. J. Barnes, London, UK). This condition is associated with increased tachykinin synthesis, decreased activity of their metabolizing enzymes and enhanced tachykinin receptor expression in the airways. Three tachykinins are known to be distributed throughout the peripheral and central nervous system. These are substance P (SP), neurokinin A (NKA) and NKB which share the common C-terminal pentapeptide sequence, Phe-X-Gly-Leu-Met-NH_2. Three receptor types have been reported, NK_1, NK_2 and NK_3; SP exhibits greater affinity for NK_1 receptors, activation of which is responsible for the inflammatory effects of tachykinins. Stimulation of NK_2 receptors, for which NKA and NKB have greater affinity, results in bronchoconstriction among other responses. A role of NK_1 receptors in asthma is suggested by the fact that NK_1R-mRNA expression is enhanced in asthmatic human lungs.

A variety of approaches have been taken to develop drugs able to modulate neurogenic inflammation. Very recently, several new non-peptide antagonists of tachykinin receptors have been described which offer new possibilities for the therapy of inflammation and pain. One of these, CP-96,345, an NK_1-selective antagonist, inhibits experimentally induced plasma exudation in the guinea-pig lung, but not bronchoconstriction. The joint NK_1/NK_2 antagonist FK224 inhibited acute bronchoconstriction and the associated cough and mucus secretion after inhalation by patients with asthma.

Another approach to therapy is to modulate tachykinin breakdown. Since compounds which induce wheezing in asthmatics inhibit neutral endopeptidase (NEP) and corticosteroids which are effective in the treatment of asthma enhance NEP activity, inhibition of SP and NKA catabolism in this way may be a promising approach.

Inhibition of the release of neuropeptides, as demonstrated by inhibition of non-adrenergic, non-cholinergic (NANC) bronchoconstriction in guinea-pigs, can be achieved with centrally-acting opioids and K^+ channel openers such as cromakalim. Given nasally, the neuropeptide depletor, capsaicin, inhibits vasomotor rhinitis in man and is administered topically in some countries as a means of local analgesia. The BK_2R-antagonist HOE 140 is also able to inhibit NANC bronchoconstriction in guinea-pigs, probably by inhibiting neuropeptide release. However, corticosteroids still provide the broadest and most effective therapy of asthma and it remains to be seen to what extent inhibition of neurogenic inflammation benefits the asthmatic patient.

CGRP, released from sensory neurones during neurogenic inflammation, is a potent and long-lasting vasodilator. It is present in enteric neurones and contributes to sensory control of gastric blood flow in response to increased gastric activity (P. Holzer, Graz, Austria). CGRP can also be released from enteric neurones by capsaicin. The action of CGRP in causing enhanced blood flow is blocked by the NO-synthesis inhibitor, L-NAME. It thus appears that gastric reactive hyperemia to acid secretion involves production by sensory neurones of CGRP which stimulates NO release from vascular endothelium leading to vasodilation.

Cell adhesion molecules in inflammation

The diapedesis of leukocytes from the blood into the tissue during immune and inflammatory responses is controlled by soluble mediators, such as cytokines and directed by adhesion molecules on the surfaces of participating cells. There are five main classes of adhesion molecules: selectins, integrins, the immunoglobulin superfamily, CD44 and cadherins. The process of diapedesis which these molecules control involves firstly, the tethering phase, secondly, the triggering of receptors on endothelial cells and thirdly, the glue phase (N. Hogg, London, UK).

The *tethering phase* is controlled by the selectins: L-selectin (Mel 14, LECAM, LAM-1), E-selectin (ELAM-1) and P-selectin (CD62, PADGEM, GMP-140). These molecules extend into the blood ready to angle a passing cell. The initial response to an inflammatory stimulus is the upregulation within a few minutes of P-selectin on endothelial cells which is rapidly shed and replaced by E-selectin. This continues to be expressed for several hours. P- and E-selectins attach to L-selectin (which is constitutively expressed) on circulating leukocytes, an interaction which leads to rolling of leucocytes along the endothelium. The interaction between the two types of selectin is mediated by sialylated Lewis-X bound to L-selectin.

A firmer adhesion of the leucocytes to the endothelium is achieved by binding of selectins to integrins, which are expressed only on cell activation (e.g. by cytokines).

CD44 on endothelial cells appears to present small

molecules to leucocytes and lymphocytes and thereby facilitates their activation.

Integrins are a very large family of heterodimers consisting of α and β sub-units, the β_2 family being exclusive to leucocytes. They include LFA-1 and Mac-1 on leucocytes, which bind to ICAM-1 and ICAM-2 on endothelial cells. Cell activation causes clustering on the cell surface of integrins such as ICAM-1 or LFA-1 with a consequent increase in their binding avidity. This only lasts for 15–100 min and then avidity and clustering decreases, thereby regulating the extent of cell contact. Integrin expression occurs only at points of cell-to-cell contact where integrins bind with their respective ligands.

A number of integrins including ICAM-1 and 2 belong to the immunoglobulin superfamily of molecules. These bind not only integrins but other molecules as well. For instance, ICAM-1 is an adhesion molecule for malaria parasites and a receptor for rhinoviruses as well as binding LFA-1. Another group of adhesion molecules, the *cadherins* form junctions between endothelial cells.

Use of antibodies and specific binding ligands has not only elucidated the mechanisms of leucocyte diapedesis but also suggested new approaches to anti-inflammatory therapy (K. Ley, Berlin, Germany). Rolling of leucocytes is inhibitable not only by antibodies to α-selectin but also by many sulphated polysaccharides (e.g. heparin). Since sialyl-Lewis-X is the natural tetrasaccharide ligand for L-selectin, it seems likely that polysaccharides can be developed which specifically inhibit this early event in diapedesis. While only specific monoclonal antibodies, such as the anti-CD18 antibody, 60.3, have so far been shown to inhibit leucocyte adherence to endothelium, collagen types II and IV have been found to inhibit Mac-1 binding and may represent natural ligands for leucocyte integrins in the extracellular space. The complement-mediated Schwartzmann reaction is also inhibitable by antibodies to CD18 (a component of both LFA-1 and Mac-1) and ICAM-1 (L. W. Argenbright, Boehringer Ingelheim, Ridgefield, USA). Immune complex-mediated vasculitis on the other hand is only inhibited by anti-CD18 antibodies not by anti-ICAM-1, indicating that binding of leucocytes to endothelium is not involved in this condition.

Future trends in anti-inflammatory therapy

The use of monoclonal antibodies for the treatment of immunological and inflammatory disorders generally is becoming continually more attractive. In a few cases of patients with autoimmune diseases (Sjögren's disease, polyarteritis and Still's disease) which were refractive to the immunosuppressant cyclosporin A and/or corticosteroids, combined treatment with anti-lymphocyte and anti-CD4 (anti-T helper cell) antibodies produced marked and prolonged remission (H. Waldmann, Cambridge, UK). This state of immunological tolerance could be induced in mice by combined treatment with a foreign antigen (e.g. human gamma globulin, HGG) and antibodies to CD4. Boosting with antigen maintained tolerance to the HGG which was mediated by $CD4^+$ T cells. Similarly, rejection of transplanted grafts could be prevented permanently by treatment with antibodies to CD4 and CD8 lymphocyte markers. The reconstitution of tolerant animals with naive lymphocytes leads to acquisition of tolerance by the transferred lymphocytes, suggesting that a soluble mediator may be involved in tolerance induction.

Another approach to anti-inflammatory therapy which is undergoing intensive investigation is the development of cytokine antagonists. The prototype is the endogenous analogue of IL1 which acts as an IL1 receptor antagonist (IL1ra). In studies on rats with streptococcal cell wall arthritis IL1ra caused 60% inhibition of arthritis and prevented cartilage erosion when given by mini-pump (R. C. Thompson, Synergen, Boulder, USA). It prevents eosinophil infiltration in guinea-pigs sensitized by aerosol antigen, inhibits mortality in graft-versus-host disease and produced 90% survival in rabbits with endotoxin shock. Preliminary results of clinical trials are particularly interesting. In rheumatoid arthritis IL1ra produced a reduction in the number of tender joints within 2 days and did not cause immunosuppression after 4 weeks of administration. Clinical trials are in progress in asthma and graft-versus-host disease and on infusion for 72 h to patients with septic shock, IL1ra produced dose-dependent inhibition of subsequent mortality (from 44%, $n=25$, on placebo to 16%, $n=25$, with 133 mg IL1ra). A large scale phase III clinical trial in septic shock ($n=1000$) is currently in progress.

In addition to the immunophilin binding drugs cyclosporin A, FK 506 and rapamycin which have been shown to inhibit cytokine release, some other low molecular weight inhibitors of cytokine release have been described recently (A. R. Mackenzie, Sandoz, Bern, Switzerland). IX 207–887 is an inhibitor of IL1 release from human mononuclear

cells and IL6 release from chondrocytes *in vitro*, but has no effect on IL1 transcription, translation or actions. It does not inhibit cyclo-oxygenase, 5-lipoxygenase or phospholipase A_2 but inhibits bone and cartilage proteoglycan loss in adjuvant arthritis in rats. Clinical studies have also indicated anti-rheumatic activity.

The phosphodiesterase inhibitor pentoxyfylline inhibits TNFα release from mononuclear cells *in vitro*, reducing circulating levels of TNFα in volunteers given endotoxin and increasing survival in septic shock. Its action *in vitro* can be mimicked by other agents causing a rise in intracellular cyclic AMP. Recently, workers at MSD have reported the structure of IL1β convertase. It's natural substrate is unique among known mammalian enzymes and the substrate analogues Ac-Tyr-Val-Lys-Asp-CHO and Ac-Tyr-Val-Ala-Asp-$CoCHN_3$ inhibit the enzyme. It seems likely that new compounds with synthesis-inhibiting activities will arise from this programme in the near future.

Postscript

This meeting gave participants a thumbnail sketch of some of the most intensively investigated current research topics. On the other hand, we were also exposed to a much more wide-ranging theme during the official buffet in the space exploration section of the Science Museum. For all their efforts we are grateful to Tim Williams and his organizing team.

This was the last EWI under the "old management". During the meeting the inaugural general meeting of the European Inflammation Society (EIS) was held and future EWIs will be organized under the aegis of the EIS, its newly elected committee and President, Kay Brune.