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Leflunomide (HWA 486), a novel immunomodulating compound for the treatment of autoimmune disorders and reactions leading to transplantation rejection

R. R. Bartlett [1], M. Dimitrijevic [2], T. Mattar [3], T. Zielinski [3], T. Germann [3], E. Rüde [3], G. H. Thoenes [4], C. C. A. Küchle [4], H.-U. Schorlemmer [5], E. Bremer [6], A. Finnegan [6] and R. Schleyerbach [1]

[1] Pharmacological Research, Hoechst AG Werk Albert, W-6200 Wiesbaden 12, FRG, [2] Immunology Research Center, Belgrade, Yugoslavia, [3] Immunology, Johannes Gutenberg University, W-6500 Mainz, FRG, [4] Medical Clinic, Ludwig-Maximilians University, W-8000 Munich 2, FRG, [5] Behring Research Laboratories, W-3550 Marburg, FRG, [6] Department of Medicine, Rush Medical Center, Chicago, Ill, USA

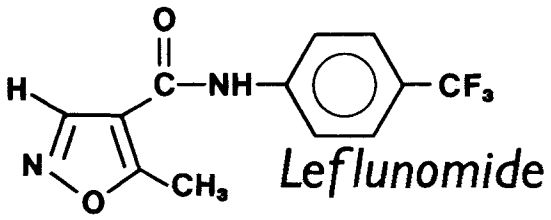
Abstract

Leflunomide has been shown to be very effective in preventing and curing several autoimmune animal diseases. Further, this agent is as effective as cyclosporin A in preventing the rejection of skin and kidney transplants in rats. Preliminary results from patients suffering from severe cases of rheumatoid arthritis demonstrated that clinical and immunological parameters could be improved with leflunomide therapy. Mode of action studies revealed that this substance antagonizes the proliferation inducing activity of several cytokines and is cytostatic for certain cell types. In this light, we could show that tyrosine phosphorylation of the RR-SRC peptide substrate and the autophosphorylation of the epidermal growth factor (EGF) receptor were, dose dependently, inhibited by leflunomide. EGF activates the intrinsic tyrosine kinase of its receptor, which stimulates the phosphorylation of a variety of peptides, the amino acid residue in all cases is tyrosine. These results indicate that much of leflunomide's activity could be due to the inhibition of tyrosine-kinase(s), which is an important general mechanism for the proliferation of various cell types. Thus, leflunomide, which is effective against autoimmune diseases and reactions leading to graft rejection, would seem to have a mode of action separating it from known immunosuppressive drugs.

Introduction

Leflunomide (HWA 486) an isoxazol derivative with antiphlogistic and novel immunomodulating properties, would seem to be a universal drug to combat autoimmune disorders [1–10]. Although the pharmacological profile of this substance has recently been reviewed [1], so much more data has been generated that a new review is warranted. Here we will briefly cover the already published results and present some new and preliminary data dealing with leflunomide's effects in animal models of autoimmunity and organ transplantation, as well as some of our most recent *in vitro* findings concerning the mode of action of its active metabolite, A771726. Further, very preliminary clinical data concerning leflunomide's effects on the immune response of patients with rheumatoid arthritis will be presented.

[1] Address for correspondence.



Autoimmune animal studies

Leflunomide inhibits the adjuvant disease of rats

Studies dealing with effects of leflunomide on the adjuvant arthritic disorder, of Lewis rats, offered us the initial clues that leflunomide may have anti-inflammatory and immunomodulating properties. Using the "standardized arthritic assay" described by Peper et al. [11] to differentiate between non-steroidal anti-inflammatory drugs and immunosuppressive agents, we found that not only was leflunomide able to arrest the development of adjuvant arthritis, but, unlike immunosuppressive agents that were considered to be exclusively active in this assay, it could restore the diminished mitogen induced lymphocyte response of the diseased animals [2]. Further, although effective in this assay, to our surprise, leflunomide did not demonstrate immunosuppressive activity, at least concerning the ex-vivo response of lymphocytes to mitogens in healthy rats [2].

These results were independently confirmed by Pasternak et al. [6], as well as Hambleton and McMahon [8]. Pasternak found further that leflunomide significantly reduced edema, fibrinogen levels, and erythrocyte sedimentation rates. The antiarthritic effects of this agent were more sustained than those observed after cyclosporin A (CSA) therapy. Whereas both leflunomide and CSA could reduce the delayed type hypersensitivity (DTH) response to mycobacterial antigen on day 9 followed by a rebound to an enhanced DTH response on day 21, only leflunomide was able to restore the suppressed mitogenic response of splenocytes to phytohemagglutinin (PHA), a T-cell mitogen. Hambleton and McMahan confirmed the suppressive effects of leflunomide and CSA on the early (day 10) DTH-reaction, with no effect, on this reaction, when tested on day 15. Similar results were observed when these animals were treated with prednisolone (PRED), whereas neither indomethacin nor tiaprofenic acid influenced the DTH reaction.

Leflunomide arrests murine systemic lupus erythematosus (SLE)-like disease of MRL/lpr-mice

SLE is an autoimmune disease 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. hypergammaglobulinaemia, and glomerulonephritis [3, 12, 13]. These mice may equally well serve as a model for human rheumatoid arthritis, especially considering the articular involvement, such as swelling of the pawns, pannus formation, proliferation of synovial tissue, degradation of articular cartilage, and the presence of circulating rheumatoid factor (RF) [3, 12–14].

Treatment of MRL/lpr mice with leflunomide dose dependently arrested disease progression and prevented the development of glomerulonephritis [3, 4]. This was due to the suppression of circulating immune complexes (Table 1), which was a direct result of the greatly lowered autoantibody formation, such as those to dsDNA or to immunoglobulins (RF) [3, 4]. Further, the tremendous number of lymphocytes which accumulate in the lymphnodes and spleens of MRL/lpr-mice could be greatly suppressed, depressing the amount of the double negative T-cells, i.e. T-lymphocytes possessing neither CD4 (T-helper cell phenotype) nor CD8 (T-suppressor cell phenotype) differential antigens (Table 1). At the same time the ratio of CD4/CD8-T-cells, which is greatly increased in these mice, was restored to normal values. These effects were also observed after treatment of these animals with CSA (Table 1). Furthermore, leflunomide therapy could restore not only the suppressed proliferative response of lymphocytes to T-cell mitogens (PHA and concanavalin A (Con A)), but also the depressed activity of macrophages to phorbolmyristenacetate (PMA) [4].

The disease inhiting effects of leflunomide were not limited to prophylactic activity [7]. MRL/lpr mice that had elevated levels of protein in their urine

Table 1
Effects of drugs on MRL/lpr-disease.

Mouse strain	Drug	Dose mg/kg/day	No. double neg. T-cells [1]	CD4/CD8 ratio	Proteinuria mg/mouse	Circulating immune complexes [2]
C3H	none	0	30	2.5	<0.1	0.1
MRL/lpr	none	0	1000	7.0	1.4	2.0
MRL/lpr	HWA486	35	100	2.0	0.1	0.5
MRL/lpr	CSA	100	50	1.9	1.7	0.5

Therapy was initiated when the animals were 10 weeks old and terminated when the animals were 22 weeks of age. The data given was obtained 6 weeks after the last drug application.
[1] Double negative T-cell are those whithout CD4 or CD8 differential antigens (double negative/lymphnode).
[2] Data is given as OD at a serum titer of 1/3200.

were aministered, for 10 weeks, either leflunomide, CSA, or prednisolone (PRED). At the end of the medication period, 90% of the animals receiving leflunomide were still alive, whereas 50% of the non-treated control mice and only 40% of the CSA or PRED medicated animals survived. Although all of the surviving mice had normal urine-protein levels, only the leflunomide medicated rodents had significantly reduced levels of RF and autoantibodies to dsDNA. CSA and PRED treatment resulted in amplified titers of autoantibody to dsDNA. It would appear that leflunomide is better suited to combat the established affliction of MRL/lpr mice than either CSA or PRED.
As to the question about what happens when leflunomide therapy is terminated after the animals have become "healthy", we have published results offering a good answer [1]. We treated MRL/lpr mice with either 35 mg/kg leflunomide or 20 mg/kg azathioprine, starting when the animals were 10 weeks old. After 9 weeks (19 weeks of age), the therapy was discontinued and the disease development followed. The progression of the ailment in animals given azothioprine could be slowed down, but, even before the therapy was terminated, the symptoms of the disorder advanced to the same level as that of non-treated MRL/lpr-mice. Leflunomide therapy, on the other hand, not only prevented the appearance of symptoms, but 20 weeks after the treatment was ended, no signs of the illness could be detected [1], although they did slowly appear somewhat later.

Leflunomide therapy prevents paralysis in experimental allergic encephalomyelitis (EAE)

EAE is a T cell mediated, neurologic autoimmune disease that develops in susceptible animals following sensitization with either spinal cord homogenate, or myelin basic protein [15]. Although the induction of EAE is essentially due to cellular immune reactions [16], there is increasing evidence for an additional role of humoral factors in the pathogenesis of this illness [17]. EAE in animals is considered to be an appropriate model for multiple sclerosis (MS) in man [18]. In Lewis rats, clinical EAE is characterized by the development of transient hindquarter paralysis [1].
Studying the effects of leflunomide on the prevention of paralysis in an acute form of EAE in Lewis rats, we found that this agent was as effective as CY [1]. Yet, contrary to the effects of CY, splenocytes from animals treated with leflunomide responded normally to T and B cell mitogens [1].

Leflunomide prevents organ specific nephritic diseases

Examples of leflunomide's effects on organ-specific autoimmunity have been very recently reported from two independent laboratories using two different animal models of nephritic disorders. Thoenes et al. [10] demonstrated that this agent is very effective in preventing experimental tubulointerstitial nephritis (TIN) in rats. TIN is induced by immunizing animals with either homologous or heterologous tubular basement membranes (TBM) in Freund's complete adjuvants. In rats, TIN commences at about 10 days after TBM (from sheep) stimulation, leading to serious damage to the kidney cortex and decreased kidney function [19]. In the above mentioned study [10], it was found that leflunomide was just as effective as CSA, but more efficacious than PRED, naproxen, or indomethacin in preventing disease development. Regarding the inhibition of autoantibody

formation to TIN, leflunomide was much more effective than the other drugs tested.
Using an antibasement membrane antibody induced glomerulonephritis in rats, Ogawa et al. [9] could show that an oral dose of 2 mg/kg/d leflunomide resulted in significant decrease in total urinary protein, plasma cholesterol and fibrogen, as well as decreased incidences of fibrin, IgG and C_3 deposits. This was the case for both preventive (two days before disease induction and ending on day 20) as well as curative (five days after induction and ending on day 20) drug therapy.

Effects of leflunomide on inflammatory and allergic reactions

Concerning antiallergic activity, we have observed that leflunomide effectively inhibits the edema formation in the skin of guinea pigs sensitized with specific IgE (passive cutaneous anaphylaxis test) [1]. Further, this drug was as effective as phenylbutazone in inhibiting the inflammatory reaction induced by carrageenan [1].

Leflunomide suppresses reactions leading to organ transplantation rejection and graft versus host diseases

Effects of leflunomide on mice undergoing a chronic graft-versus-host (CGVH) reaction

The intravenous injection of a mixture of parental splenocytes into healthy inbred F_1-mice 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 (murine H_2-antigens) 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 non host recognition of donor. The ensuing immune abnormalities depend on the parental and F_1 strain combinations used. For example, the inoculation of C57BL/6 spleen cells into (C57BL/6 × DBA/2) F_1-mice, further referred to as DF_1-mice, leads to the development of an acute GVH (AGVH))-disease resulting in profound immunodeficiency, anemia, hypogammaglobulinemia, the appearance of suppressor cells [20] and the development of cytotoxic T-lymphocytes (CTL) specific to BDF_1-alloantigens [21]. In contrast, inoculation of DBA/2 cells into BDF_1-mice results in a chronic GVH (CGVH)-reaction in which lymphoid hyperplasia, autoantibody production, immune complex glomerulonephritis [21, 22] and the failure to form CTL to BDF_1-alloantigens [20], i.e., an illness resembling human systemic Lupus erythematosus (SLE).
First, we studied the effects of leflunomide on the chronic graft versus host (CGVH) disease of mice, i.e. animals undergoing a disease displaying symptoms very similar to SLE. Comparing the protective effects of this agent to those of CY, PRED, and indomethacin, we found that when therapy was started 4 weeks after disease induction (shortly before the first appearance of proteinuria), only indomethacin was ineffective in inhibiting the SLE-like symptoms [5]. Curiously, although PRED could prevent the development of glomerulonephritis and thus proteinuria, it did not inhibit the deposition of immune complexes on the glomeruli [5]. This may be due to the mode of action of steroids, which have been reported to inhibit complement, as well as the production of interleukin-1 (IL-1) [1]. Interestingly, although leflunomide is not a cytotoxic agent, as is CY [23], and to some extent PRED, the splenomegaly of the CGVH-diseased mice was dose-dependently inhibited after therapy with this agent [5].
As the case in both adjuvant- and MRL/lpr-diseased animals, mice undergoing a CGVH-reaction have significantly suppressed lymphocyte responses to T cell mitogens (Con A and PHA). Treatment with leflunomide restored these responses, whereas neither indomethacin nor PRED displayed any positive effects. Depending on the dose, CY partly restored or inhibited these mitogen induced responses of T cells [5, 23].

Prevention of skin and kidney graft rejection by leflunomide

In the prevention of reactions leading to transplant rejection, leflunomide initially seemed to be completely ineffectual [1]. Although successful in preventing the chronic graft-versus-host (CGVH)-disease [5], this agent was first reported not to have any protective activity in the runting illness brought on by an acute GVH reaction [1]. Due to the results obtained from the effects of this agent on the murine CGVH-disease, and considering that all studies we [1–7] and others [6, 8, 9] had

Table 2
Effects of drug therapy on allogenic kidney transplantation.

Transplant	*n*	Drug	Dosage (mg/kg/d)	Plasma-creatine levels on day (mg/dl ± SD)					Survival rate (d ± SD)
				8	32	40	50	60	
Syngenic	7	none	0	0.7± 1.2	0.6±0.4	0.6±0.3	0.6±0.2	0.6±0.3	>60
Allogenic	6	none	0	7.0±16.1					8.0±0.3
Allogenic	12	HWA486	5 (po)	0.7± 0.7	0.7±0.8	0.7±0.6	0.7±0.3	0.7±0.3	>60
Allogenic	13	HWA486	10 (po)	0.7± 0.7	0.7±0.7	0.7±0.7	0.7±0.8	0.7±0.8	>60
Allogenic	13	CSA	10 (po)	0.8± 0.4	0.7±0.5	0.7±0.8	0.7±0.9	0.7±0.5	>60
Allogenic	7	AZA	5 (iv)	7.9±17.4					8 ±0
Allogenic	7	PRED	5 (iv)	6.9± 4.6					7.9±0.6

Lewis rats were transplanted with either syngenic (Lewis) or allogenic (BN) kidneys. Drug therapy was initiated on day −1 and terminated on day 30 as indicated. AZA = azathioprine; CSA = cyclosporin A; PRED = prednisolone; HWA486 = leflunomide; po = per os; iv = interveinous. From Küchle et al. [24].

Table 3
Effects of leflunomide therapy on allogenic skin transplantation.

Transplant	*n*	Dosage (mg/kg/d)	Graft survival time (d/SD)
DA→LEWIS	10	0.0	10.5±1.1
DA→LEWIS	10	2.5	19.7±1.9
DA→LEWIS	10	5.0	23.7±2.0
DA→LEWIS	10	10.0	27.0±1.1
DA→LEWIS	10	20.0	29.1±1.8
LEWIS→FISHER	10	0.0	16.2±1.0
LEWIS→FISHER	10	2.5	22.6±2.2
LEWIS→FISHER	10	5.0	25.9±2.1
LEWIS→FISHER	10	10.0	28.9±1.7
LEWIS→FISHER	10	20.0	33.8±2.8

Rats were treated from day 1 to 10 with leflunomide (per os) after tail skin was transplanted. From Küchle, et al. [24].

conducted demonstrated that leflunomide was just as effective as CSA in the therapy of various autoimmune disorders, we reasoned that this drug must also be efficient in preventing transplantation rejection reactions.

Using Lewis rats (RT 1I), as host animals, kidneys from BN rats were transplanted. In the untreated rats, these allografts were rejected within eight days, whereas treatment with leflunomide, for 30 days, prolonged the graft and thus the animal survival of all of these animals for the duration of the experiment (more than 60 days) (Table 2). Following the serum-creatinine levels we could determine that the transplanted kidneys functioned normally (Table 2), and the histological studies revealed virtually no signs of chronic rejection [24]. The results we obtained from CSA therapy were very similar to those observed after leflunomide, whereas, in our experiment, neither azathioprine nor prednisolone offered any protection (Table 2).

Looking further, we found that leflunomide was not only efficacious in suppressing kidney but also skin rejection reactions in rats. Using two different strain combinations for our studies, DA/Lewis (MHC and non-MHC different) and Lewis/Fisher (non-MHC different), tail skin from the donor animals was grafted to the hosts. Therapy with leflunomide was started one day after transplantation and terminated on day ten. Using this protocol, a dose dependent depression of the rejection time could be observed in both transplant combinations (Table 3). With this protocol, i.e. starting drug application a day after exposure to foreign antigen, CSA is not efficient, at least in our hands. This is because CSA is much better suited to suppress primary reactions, before they are initiated, and is much less efficient in inhibiting ongoing immune reactions [1, 7, 25]. This indicates a much different mode of action of leflunomide than that of CSA.

Studies concerning the mode of action of leflunomide

Ex vivo and in vivo studies

For a long time, we felt that leflunomide did not have any or very little influence on T-cells. It seemed that this drug asserted its effects chiefly on B-cells, or perhaps T-cell products mediating B-cell activity. This was based on our findings that

leflunomide seemed to be only effective in diseases in which primarily B-cells and autoantibodies played a major role, i.e. SLE, etc., but did not seem to be effective in AGVH-reactions, which is mainly T-cells mediated. That the ineffectiveness of leflunomide in the murine AGVH-disease was a matter of dosage, we discovered later (Bartlett, unpublished results). Of course, SLE and other autoimmune diseases are T-cell mediated, thus we could have realized much earlier that this agent must assert some effects on these lymphocytes. That T-cells are affected by this agent has become very obvious from our results concerning transplantation rejection. Perhaps one of the primary, at the time misleading, results originated from the ex-vivo experiments using healthy mice [26]. Comparing the effects of leflunomide therapy on the immune response to those of cyclophosphamide (CY), prednisolone (PRED), and CSA, we found that like the other three agents, leflunomide arrested the development of antibody producing cells to the T-cell antigen SRBC, and, thus, suppressed the production of their specific antibody. Yet, unlike CY and PRED, neither CSA nor leflunomide had inhibitory effects on the proliferative response of lymphocytes to T-cell independent B-cell mitogens, i.e. lipopolysaccharide (LPS) and dextransulfate (DXS). Contrary to the activities of the other drugs, leflunomide did not inhibit the response of lymphocytes to the T-cell mitogens concanavalin A (Con A), phytohemagglutinin (PHA), nor, unexpectedly, to the preimmunized T-cell antigen sheep red blood cells (SRBC). The reaction to SRBC was unanticipated, because of the lack of antibody response to this specific antigen, yet the proliferative response was still detectable. Further, not only was the oxidative burst of peritoneal macrophages, after PMA induction, not inhibited, as was the case after treatment with the three immunosuppressive substances, but this response was greatly enhanced after therapy with leflunomide [26].

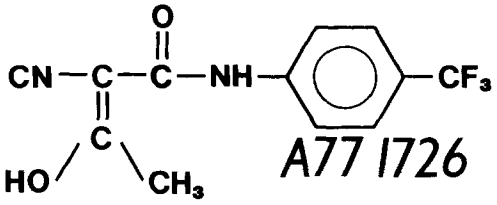
These results suggested that leflunomide has, on the one hand, suppressive activity on the development of T-lymphocyte dependent antibody producing cells and the formation of their specific antibody, yet, on the other hand, does not interfere with the proliferative response of lymphocytes to this antigen. Due to the fact that this agent did not inhibit T-cell responsiveness to mitogens, it seemed that leflunomide may influence, somehow, the T-B lymphocyte cooperation, without negatively influencing general T-cell or other leukocyte cooperation and function. That this interpretation was not fully correct became obvious later when we obtained data indicating that T-cells are most likely inhibited in their activity, i.e. inhibitory effects of leflunomide on T-cell mediated DTH-reaction to mycobacterial antigens of rats with adjuvant arthritis [6, 8,], and suppression of skin and kidney transplantation rejection was observed [24].

In vitro studies

For the *in vitro* studies, the primary metabolite of leflunomide, A771726 was used. This metabolite is the molecule, which is very stable and makes up more than 90% of compound found in serum of animals and humans and is responsible for the disease modifying activity of this drug. This has been determined in the adjuvant arthritis-disorder, MRL/lpr-disease, and the murine CGVH-illness [1].

Effects on mediator release of inflammatory cells

Hypersensitivity reactions are inflammatory responses resulting from the release of mediators from tissue mast cells or basophilic leukocytes. In allergic individuals, this release is initiated by an antigen (in this case allergen) cross-linking two IgE molecules affixed to their receptors in the cell's plasma membrane. Mast cells and basophils also have receptors for other immunoglobulins, i.e. IgG_1, which mediate anaphylactic responses with a short sensitization period (not more than a few hours). Not only immunologic induction of mediator release is possible, but also non-immunologic means can lead to such liberation, for instance calcium ionophores [27], and basic polypeptides [28] have been reported to provoke the discharge of mediators from mast cells.



An important primary mediator of hypersensitivity is histamine, which binds to target cells (smooth muscle cells, endothelial cells of blood vessels) via specific receptors. We have found that leflunomide's primary metabolite, A771726, can inhibit the release of histamine from isolated human basophils, rat peritoneal mast cells, and a murine bone marrow derived mast cell line [1]. This is true for both calcium-ionophore (A23187) and IgE induced release. The ID_{50} values, for both types of induction, were found to be about 0.7 μM [1].

Not only histamine release, but also the formation and liberation of biologically active metabolites of arachidonic acid were shown, to varying degrees, to be inhibited by leflunomides's primary metabolite. For example, the calcium-ionophore induced release of 5-HETE was strongly inhibited ($ID_{50} \approx$ 3 μM), leukotriene B_4 (LTB_4) considerably less suppressed ($ID_{50} \approx 100$ μM) and PGE_2 formation was not affected [1]. Despite the limited ability to inhibit the generation of mediators from arachidonic acid, antagonism of contractive activity of one such mediator, prostaglandin $F_2\alpha$ ($PGF_2\alpha$), was detected [1]. Further, we have observed that the generation of oxygen radicals, during mediator liberation, can be effectively prevented by treatment with leflunomide or its primary metabolite, A771726 [1].

Taken together, many of the antiphlogistic effects of leflunomide observed in animal models surely have to do with the activity discovered *in vitro* on various isolated inflammatory cells and their mediators. Perhaps, also, these effects may explain some of the immunorestoring characteristics of this agent. For instance, it has been shown that LTB_4 has many effects on various immune cells, including T-lymphocytes and natural killer function [29].

Effects on lymphocytes and cytokines

Due to leflunomide's obvious immunomodulating properties, our interest has focused on its influence on various mediators of immunocompetent cells. As already mentioned, we could demonstrate that T-dependent antibody production of healthy mice [26], and the formation of self-reactive antibodies (T-dependent) in autoimmune animals, can be effectively suppressed, both preventively and curatively, by oral treatment with this drug [1–9]. Further, disorders requiring T-T cell cooperation, i.e. transplantation rejection, could be inhibited by leflunomide therapy. Thus, it was our feeling that leflunomide must somehow interfere in the interaction of lymphocytes, especially T and B cells, and that this interference is not limited to primary immune responses, but is effective on secondary immune reactions as well.

We first studied the effects of leflunomide's primary metabolite (A771726) on the *in vitro* PFC-assay, to determine if we could observe the same effects we were seeing *in vivo*. We found that this molecule could dose dependently block the formation of PFC to the T cell dependent antigen SRBC, even when it was given into the culture as late as 4 days after they were set up and assayed on day 5 [1]. This paralleled our experience in animal experiments [1]. This inhibition of PFC-formation could, though, be partially overcome if fairly high concentrations of Con A supernatant (containing several T-cell growth factors) were added into the cultures on day 2 [1]. This data indicated that something in Con A supernatant could replace what leflunomide had somehow blocked. This could be either through inhibition of mediator production or interference of their activity.

Our interest then fixed on leflunomide's effects on cytokine production. Confirming and adding to our already published results concerning IL-1, IL-2, IL-3 production [1], Germann, et al. (manuscript in preparation) have found that the formation and release of cytokines formed by either Th_1 or Th_2 cells, i.e. IL-1, IL-2, IL-3, IL-4, TRF-α, or IFN-γ, are not inhibited by this agent. Thus, if this drug does have effects on cytokines, then it must somehow interfere in their activity.

The first evidence that leflunomide does antagonize T cell products was obtained in an assay system for T helper cell replacing factor (TRF) [30]. Athymic lymphocytes from nu/nu mice are unable to generate plasma cells producing antibody to sheep red blood cells (SRBC) because they lack T lymphocytes. It is possible to restore this humoral response by replacing helper cell function through the addition of T cell products that act directly on B cells. When supernatant from Con A stimulated T cells, containing TRF, is given into a culture of nu/nu-splenocytes and SRBC on day 2, large numbers of PFC to this antigen can be detected on day 5. When A771726 was given into such cultures on day 0 or 2, a dose dependent inhibition of specific plaque formation could be observed [1].

The activity of TRF has been attributed to interleukin 5 (IL-5), yet, as tested in the above described system, it is difficult to ascribe these results using Con A supernatant to one interleukin alone, because this activity is most likely resulting from a combination of distinct factors interacting with each other.

With the coming of recombinant interleukines, it has become possible to study these individually. Leflunomide seems, to varying degrees, to interfere in the activities of several interleukines. We have found, for instance, that IL-3 induced proliferation can be strongly antagonized by A771726 [1], with an ID_{50} of about 3 μM. IL-3 is a sort of universal colony stimulating factor with varying effects on several haematopoietic cells, i.e. the activity attributed to such factors as mast cell growth factor (MCGF), P-cell stimulating factor (PSF), burst promoting activity (BPA), haematopoietic growth factor (ECSF), megakaryocyte colony stimulating factor (MEG-CSF), and eosinophile colony stimulating factor (Eo-CSF). IL-3 has been reported to support the growth of murine pre-B cell clones [31], enhance immune responses to T cell-dependent antigens [32], and strongly inhibit the generation of NK cells *in vivo* [33]. Recently, it has been demonstrated that the sera of autoimmune MRL/lpr mice contain antibodies against the IL-3 receptor, inducing IL-3 like activity, which may play an important role in the pathology of these animals.

Yet, not only is IL-3 strongly antagonized, but also IL-4 to almost exactly the same degree [1]. IL-4 (BSF-1) also exerts multiple biological activities that affect various cell of hematopoietic lineages. In their review, Paul and Ohara [34] propose a principal role for IL-4 in the response of B cells to T cell dependent antigens. Further, it is known that this interleukine promotes the formation of IgG_1 and IgE antibodies. To a lesser extent than IL-3 or IL-4 is the activity of IL-2 antagonized ($ID_{50} \approx 50$ μM). Of the cytokines thus far tested, only IL-1 and gamma-interferon are not affected in their activity by leflunomide. Only at concentrations above 500 μM of A771726 was any antagonistic effect on IL-1 dependent IL-2 production observed, although the IL-1 dependent proliferative response of thymocytes could be inhibited at relevant concentrations ($ID_{50} \approx 30$ μM). This, though, was most likely due to antagonistic effects on IL-2, which is ultimately responsible for the proliferation of these cells [35].

We now have data showing that the cytokines G-CSF, GM-CSF and TNF-α are also antagonized by A771726 (Germann et al., manuscript in preparation). Thus, a principal portion of leflunomide activity could be explained through its antagonism of various cytokines.

Antiproliferative effects of leflunomide

From the above data, concerning leflunomide's antagonistic activity on various lymphokines, it became obvious to us that this agent does not

Table 4
Cells tested with A771726 in proliferation assays.

Cells	Origin	ED_{50}
T-cell lines:		
CTLL	Mouse T-cell line (T_C-Clone IL-2)	40–50 μM
HT-2	Mouse T-cell line (IL-2)	40–50 μM
CTL-J-K	Mouse T-cell line (T_C, IL-2)	40–50 μM
C1 9/4	Mouse T-cell line (IL-4 dep., TH_2)	25 μM
K III 5	Mouse T-cell line (IL-2, TH_1)	1–3 μM
G53	Mouse T-cell clone	30 μM
Spleen T	Mouse (ConA and PWM)	10 μM
B-cell lines:		
Spleen B	Mouse (LPS)	10 μM
A20 2J	Mouse B cell tumor (BALB/c)	1–3 μM
TRK 4	Mouse B cell hybridoma	5 μM
TRK 5	Mouse B cell hybridoma	5 μM
7 TD 1	Mouse B cell hybridoma (IL-6)	10 μM
WEHI-279	Mouse early B cell lymphoma	1 μM
7D4	Rat hybridoma	1 μM
Others:		
Bone marrow	Mouse (M-CSF, GM-CSF)	5 μM
P 388 D1	Mouse MΦ tumor	10 μM
PB-3C	Mouse mast cell line (IL-3)	20 μM
DA-1	Mouse promyelomonocytic progen. (IL-3)	5 μM
A431	Human epidermoid carcinoma	15 μM
KB	Human epidermoid oral carcinoma	15 μM
KB 3-1	Clone with low MDR activity	30 μM
KB 8.5.11	Clone with high MDR activity	30 μM
HFF	Human foreskin fibroblasts	40 μM
HL-60	Human promyelomonocytic leukemia	25 μM
Peripheral blood: human		
	with ConA	40–50 μM
	with PHA	40–50 μM
	with PWM	30 μM

specifically inhibit a discrete receptor for a certain lymphokine, but more likely a generalized mechanism common for signal transmission of these cytokines. Due to the fact that we employed bioassays in which proliferation was determined for lymphokine dependency. We decided to investigate the antiproliferative activity of leflunomide's primary metabolite. In Table 4 an overview of the various cells that have been tested with A771726 is given. From this it is obvious that this metabolite has a broad inhibitory activity on several cell types and is not species specific nor limited to immune cells. There was, however, a wide range of sensitivity to this drug. Generally, the most sensitive of these cells were of B-cell origin (lymphocyte lines and hybridomas), which, with one exception, were about ten to 40 times more sensitive than T-cell lines. The antiproliferative effects were, though, not cytotoxic and completely reversible, i.e. simple washing of the cells was enough to allow the cells to resume proliferative activity (Mattar et al., manuscript in preparation). Leflunomide is, thus, a cytostatic agent. These results may also explain the *ex-vivo* results concerning mitogen and antigen induced lymphocyte proliferation. *In vivo,* these cells are, most likely, suppressed in their proliferative response, but as soon as they are washed free of substance and placed in culture, they can react normally to stimulation. That normal reactivity, which could be observed, has also to do with a reregulation of the immune response in the autoimmune diseased individuals.

Leflunomide inhibits tyrosine kinase

We have recently initiated investigations concerning tyrosine kinase inhibition as a hypothetical mechanism for the non-cytotoxic and reversible antiproliferative activity of A771726. That this enzyme system was selected had very little to do with certain structural similarities between A771726 and the amino acid tyrosine, but much more with a receptor associated second messenger system that could provide a conceptual mechanism for growth stimulation.

The data assembled to date do indeed offer support for the idea that tyrosine kinase inhibition may be responsible for leflunomide's antiproliferative action. Mattar et al. (manuscript in preparation) have found that the autophosphorylation of the epidermal growth factor (EGF)-receptor in living cells could be inhibited by A771726. The EGF-receptor is a transmembrane glycosylated protein of 170 kDA consisting of 1186 amino acids. This molecule has an extracellular EGF binding domain and an intercellular intrinsic protein tyrosine kinase domain which catalyzes EGF-dependent tyrosine phosphorylation of various protein substrates, as well as autophosphorylation of the EGF receptor [36, 37].

The characterization of the EGF-receptor has been facilitated by the use of a human carcinoma cell line (A-431) which overexpresses EGF-receptors 20–50 fold [37]. Employing this cell line, we conducted two different types of experiments. In the first we investigated the antiproliferative effects of leflunomide's primary metabolite on the proliferative effects of the cells in the presence of EGF. Here we found that the ID_{50} was about 15 μM of A771726 (Mattar et al., manuscript in preparation).

In the second type of experiment, we prepared membrane fractions and used the artificial peptide RR-SRC (Penisula Lab., USA) with a molecular weight of 1519.86 and the sequence: Arg-Arg-Leu-Ile-Glu-Asp-Ala-Glu-Try-Ala-Ala-Arg-Gly [37]. This peptide, which is homogenous to a part of the catalytic domaine of the SRC oncogene product, contains the sequence that is autophosphorylated in the scr-gene product. Using this tyrosine phosphorylation assay system, we have found that the ID_{50} of A771726 is about 150 μM, or about 10 times that necessary to inhibit cell proliferation (Mattar et al., manuscript in preparation). Considering that we need considerably more than 10 times as many cells to obtain the necessary membrane preparation for this assay, the data seems to fit nicely.

Another cell line that is dependent on EGF is HFF (human foreskin fibroblasts) which has a normal amount of EGF-receptors and, thus, can be used as a model for normal situations. These cells are inhibited 50% in their proliferative response to EGF at a concentration of A771726 of about 40 μM. Preliminary experiments, using Western blots, reveal a dose dependent decrease in antiphosphotyrosine antibody binding to the autophosphorylated tyrosine residues of the EGF-receptor of these cells (Mattar et al., manuscript in preparation).

Taken together, these experiments strongly indicate that tyrosine kinase is inhibited through the

active metabolite of leflunomide, and this inhibition could be responsible for the suppressive activity of this agent on lymphocytes and other cell types.

Clinical effects of leflunomide on patients with RA: Preliminary immunological data

Due to the already mentioned effects on the various animal models of autoimmunity, and results from tolerance studies in healthy human volunteers (unpublished data), we decided to investigate the effects of leflunomide on patients suffering from rheumatic disorders. In a randomized, open clinical study, patients, fulfilling the criteria of the American Rheumatoid Association for severe rheumatoid arthritis, were divided into three groups ($n=7$) and treated, six weeks long, with 5, 10 or 25 mg of leflunomide. Although designed primarily to determine tolerability for a yet to be conducted long term study, we also decided to determine clinical and immunological parameter before and after drug treatment.

The results we have to date are very preliminary, but, we feel, impressive. The drug, even at the highest dose level, was very well tolerated. No toxic signs could be detected in any of the patients. Along with clinical improvements (objective joint assessment and subjective assessment by the patients), the immunological parameters were largely improved. This included reduction in the erythrocyte sedimentation rate, amount of circulating immune complexes, and autoantibody titers (RF, type II collagen). Further, almost all of the patients in the 5 and 10 mg groups developed increased numbers of T and B cells, whereas those in the 25 mg group had significantly lowered numbers. The generally elevated CD4/CD8 (helper/suppressor) T-cell ratios of the patients, in all groups, were largely reduced, whereas the number of resting B-cells (CD21) were increased, a sign of diminished immunologic activity. Additionally, as we had seen in autoimmune diseased animals, the proliferative response of lymphocytes to mitogens (PHA, Con A, and PNA) was largely restored. The clinical and immunological improvements were dose dependent, with the 25 mg dosage giving, seemingly, optimal effects.

These encouraging results have motivated us to conduct further clinical studies with leflunomide, which we will shortly initiated.

Concluding remarks

Leflunomide is an antiphlogistic and immunomodulating agent that has been shown to be efficacious in preventing and healing autoimmune disorders and reactions leading to organ transplantation rejection. From our preliminary clinical data, we now have hope that these effects, observed in animals, can truly be transferred to humans.

Although we are far from understanding the mode of action of leflunomide, we are slowly gathering some insight. In our last review we wrote that the most important aspect of this substance seemed to be its effect on B cells by interfering in functions of certain T cell products [1]. This is still an important aspect, especially considering leflunomide's activity on autoimmune diseases, but now we know that this drug does have profound effects on T-cells and that this facet is just as important, if not more so, in respect to these disorders.

A good many of the immunosuppressive effects of leflunomide could be attributed to the antagonistic effects it has on many cytokines. Through interfering in the activity of IL-2 and IL-4, the inhibition of transplantation rejection could be explained, i.e. the interference of cytotoxic T-cell formation. Considering, further, that increased IL-3 like activity has been reported in autoimmune MRL/lpr mice [45], and it is felt that this amplified activity may contribute to the pathology of their illness [45], then the interference of leflunomide with this interleukine may, together with the antagonistic activity of TRF and, specifically, IL-4, explain some of its disease modifying properties in animals with SLE-like and other autoimmune diseases. Also, IL-6 interference (Germann et al., unpublished results) could be responsible for the suppression of late phase proteins observed in adjuvant diseased rats [6] and RA-patients treated with leflunomide.

Our date to date concerning tyrosine kinase inhibition as a hypothetical mechanism for the non-cytoxic and reversible antiproliferative activity of A771726 is, granted, preliminary but, in many ways, convincing. First of all, many known receptors for growth factors are associated with tyrosine kinase, i.e. EGF [37], IL-2 (the high binding, 75 kD chain) [39], IL-3 [40], G-CSF, GM-CSF and TNF-α [41]. Leflunomide antagonizes all of these mediators. On the other hand, IL-1, which is not antagonized by leflunomide, is not associated with ty-

rosine kinase, but with threonine and serine kinase [42]. Further, considering that certain metabolites of arachidonic acid are inhibited by leflunomide, and it has been reported that the tyrosine kinase activity of the EGF-receptor is necessary for phospholipase A_2 activation [43], then may antiinflammatory effects of leflunomide could be a result of tyrosine kinase inhibition.

Another important aspect of this drug is its inhibitory effect on the release of histamine, from basophils and mast cells, because of its role in inflammatory reactions. Relating to our findings on leflunomide's activity on murine SLE-like disorders, it has been recently reported that SLE patients often exhibit abnormal production of antibodies to IgE, and that these autoantibodies may, by activating mast cells and basophils, play a consequential part in the release of vasoactive amines, thus leading to generalized tissue injury [44].

Certainly, the effects leflunomide has on the formation of arachidonic acid metabolites, as well as their function, also play an important role in its antiphlogistic activity.

Not only are we sure that leflunomide will prove to be an effective drug to combat human autoimmune disorders (evidence for this we already have), but, from studying its effects on humans and animals suffering from autoimmune diseases, we became convinced that this drug will definitely provide a tool in gaining new insights into the mechanisms leading to such ailments and, perhaps, as such an instrument, will be able to aid in finding more proficient drugs or other means to modulate these malfunctioning immune reactions.

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References

[1] R. R. Bartlett, T. Mattar, U. Weithmann, H. Anagnostopulos, S. Popovic and R. Schleyerbach, *Leflunomide (HWA 486): a novel immunorestoring drug.* In *Therapeutic approaches to inflammatory diseases* (Eds. A. J. Lewis, N. S. Doherty and N. R. Ackerman), Elsevier Science Publishing Co., Inc. New York, pp. 215–228 (1989).

[2] R. R. Bartlett and R. Schleyerbach, *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. Immunopharmac. *7,* 7–18 (1985).

[3] R. R. Bartlett, R. X. Raiss, S. Popovic and R. Schleyerbach, *Immunologic, histologic and ultrastructural studies in autoimmune MRL/1 mice.* In *Articular cartilage biochemistry* (Eds. K. E. Kuettner, R. Schleyerbach and V. C. Hascall), pp. 391–412 (1986).

[4] S. Popovic and R. R. Bartlett, *Disease modifying activity of HWA 486 on the development of SLE in MRL/1-mice.* Agents and Actions *19,* 313–314 (1986).

[5] S. Popovic and R. R. Bartlett, *The use of the murine chronic graft versus host (CGVH) disease, a model for systemic lupus erythematosus (SLE), for drug discovery.* Agents and Actions *21,* 284–286 (1987).

[6] R. D. Pasternak, N. S. Wadopian, R. N. Wright, P. Siminoff, J. A. Gylys and J. P. Buyniski, *Disease modifying activity of HWA 486 in rat adjuvant-induced arthritis.* Agents and Actions *21,* 241–243 (1987).

[7] R. R. Bartlett, S. Popovic and R. X. Raiss, *Development of autoimmunity in MRL/lpr mice and the effect of drugs on this murine disease.* Scand. J. Rheumatol. Supp. *75,* 290–299 (1988).

[8] P. Hambleton and S. McMahon, *Drug actions on delayed-type hypersensitivity in rats with developing and established adjuvant arthritis.* Agents and Actions *29,* 338–332 (1990).

[9] T. Ogawa, M. Inazu, K. Gotoh and S. Hayashi, *Effects of leflunomide on glomerulonephritis induced by antibasement membrane antibody in rats.* Agents and Actions, in press (1990).

[10] G. H. Thoenes, T. Sitter, K. H. Langer, R. R. Bartlett and R. Schleyerbach, *Leflunomide (HWA 486) inhibits experimental autoimmune tubulointerstitial nephritis in rats.* Int. J. Immunopharmac. *11,* 921–929 (1989).

[11] R. Peper, B. Alvarez, C. Colombo and H. Schroder, *The use of a standardized adjuvant arthritis assay to differentiate between anti-inflammatory and immunosuppressive agents.* Proc. Soc. exp. Biol. Med. *137,* 506–512 (1971).

[12] M. Hang, A. N. Theofilopoulos and F. J. Dixon, *A spontaneous rheumatoid arthritis-like disease in MRL/1 mice.* J. of Exp. Med. *155,* 1690–1701 (1982).

[13] A. N. Theofilopoulos and F. J. Dixon, *Etiopathogenesis of murine SLE.* Immunological Review *55,* 179–216 (1981).

[14] H. G. Fassbender, *Joint destruction in various arthritic diseases.* In *Articular cartilage biochemistry* (Eds. K. E. Kuettner, R. Schleyerbach and V. C. Hascall) pp. 371–389 (1986).

[15] D. E. McFarlin, S. E. Blank, R. F. Kibler, S. McKneally and R. Shapira, *Experimental allergic encephalomyelitis in the rat: response to encephalitogenic proteins and peptides.* Sciences *179,* 478–483 (1973).

[16] F. Mokhtarian, D. E. McFarlin and C. S. Raine, *Adoptive transfer of myelin basic protein-sensitized T cells produces chronic relapsing demyelinating disease in mice.* Nature *309,* 356–358 (1984).

[17] H. Lassmann, G. Suchanek, K. Kitz, H. Stemberger, B. Schwerer and H. Bernheimer, *Antibodies in the pathogenesis of demyelination in chronic relapsing EAE (cr-EAE).* In *Experimental allergic encephalomyelitis: a useful model for multiple sclerosis* (Eds. E. C. Alvord, M. W. Kies and A. J. Suckling) pp. 165–170 Alan R. Liss, Inc., New York (1984).

[18] E. C. Alvord, *The challenge: How good a model of MS is EAE today?* In *Experimental allergic encephalomyelitis: a useful model for multiple sclerosis* (Eds. E. C. Alvord, M. W. Kies and A. J. Suckling),pp. 3–5 Alan R. Liss, Inc., New York (1984).

[19] G. H. Thoenes, T. Umscheid, T. Sitter and K. H. Langer,

Cyclosporin A inhibits autoimmune experimental tubulointerstitial nephritis. Immunology Letters *15,* 301–306 (1987).

[20] E. Gleichmann, E. H. Elven and J. P. W. Veen, *A systemic lupus erythematosus (SLE)-like disease in mice induced by abnormal T-B cell cooperation. Preferential formation of autoantibodies characteristic of SLE.* Eur. J. Immunol. *12,* 152–159 (1982).

[21] C. S. Via, S. O. Sharrow and G. M. Shearer, *Role of cytotoxic T lymphocytes in the prevention of Lupus-like disease occuring in a murine model of graft-vs-host disease.* J. Immunol. *139,* 1840–1849 (1987).

[22] R. C. Kuppers, T. Suiter, E. Gleichmann and N. R. Rose, *The induction of organ-specific antibodies during the graft-vs-host reaction.* Eur. J. Immunol. *18,* 161–166 (1988).

[23] R. R. Bartlett, *Cyclophosphamide.* In *The pharmacology of lymphocytes* (Eds. M. A. Bray and J. Morley), pp. 453–469 Springer-Verlag, Berlin, Heidelberg, New York, (1988).

[24] C. C. A. Küchle, G. H. Thoenes, K. H. Langer, H. U. Schorlemmer, R. R. Bartlett, and R. Schleyerbach, *Prevention of kidney and skin graft rejection in rats by leflunomide, a new immunomodulating agent.* Transp. Proc. (in press).

[25] D. Bunjes, C. Hardt, M. Röllinghoff and H. Wagner, *Cyclosporin A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin 1 and interleukin 2.* Eur. J. Immunol. *11,* 657–661 (1981).

[26] R. R. Bartlett, *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. Immunopharmac. *8,* 199–204 (1986).

[27] M. Ennis and F. L. Pearce, *Differential reactivity of isolated mast cells from the rat and guinea pig.* Eur. J. Pharmacol. *66,* 339–343 (1980).

[28] M. Ennis, F. L. Pearce and P. M. Weston, *Some studies on the release of histamine from mast cells stimulated with polylsine.* Br. J. Pharmacol. *70,* 329–332 (1980).

[29] J. S. Goodwin, *Regulation of T cell activation by leukotriene B4.* Immunol. Res. *5,* 233–248 (1986).

[30] A. Schimpel, L. Hübner, C. A. Wong and E. Wecker, *Distinction between T helper cell replacing factor (TRF) and T cell growth factor (TCGF).* Behring Inst. Mitt. *67,* 221–225 (1980).

[31] R. Palacios, G. Henson, M. Steinmetz and J. P. Kearn, *Interleukine-3 supports growth of mouse pre-B-cell clones in vitro.* Nature *309,* 126–131 (1984).

[32] M. Kimoto, V. Kindler, M. Higaki, C. Ody, S. Izui and P. Vassalli, *Recombinant murine IL-3 fails to stimulate T or B lymphopoiesis in vivo, but enhances immune response to T cell-dependent antigens.* J. Immunol. *140,* 1889–1894 (1988).

[33] T. Kalland, *Physiology of natural killer cells in vivo regulation of progenitors by interleukin 3.* J. Immunol. *139,* 3671–3675 (1987).

[34] W. E. Paul, J. Ohara, *B cell stimulatory factor-1/interleukin 4.* Ann. Rev. Immunol. *5,* 429–459 (1987).

[35] H. Wagner, C. Hardt, K. Heeg, K. Pfizenmaier, W. Solbach, R. R. Bartlett, H. Stockinger and M. Röllinghoff, *T-T cell interactions during cytotoxic T lymphocyte (CTL) responses: T cell derived helper factor (interleukin 2) as a probe to analyze CTL responsiveness and thymic maturation of CTL progenitors.* Immunol. Rev. *51,* 215–255 (1980).

[36] Y. Yarden and A. Ullrich, *Growth factor receptor tyrosine kinases.* Annu. Rev. Biochem. *57,* 443–478 (1988).

[37] M. D. Waterfield, *Epidermal growth factor and related molecules.* Lancet, 1243–1246 (1989).

[38] L. J. Pike, *Assay of growth factor-stimulated tyrosine kinases using synthetic peptide substrates.* Methods Enzamol. *146,* 353–363 (1987).

[39] G. B. Mills, C. May, M. McGill, M. Fung, M. Baker, R. Sutherland and W. C. Greene, *Interleukin 2-induced tyrosine phosphorylation. Interleukin 2 receptor β is tyrosine phosphorylated.* J. Biol. Chem. *265,* 3561–3567 (1990).

[40] S. L. Pelech, H. B. Paddon, D. L. Charest and B. S. Federspiel, *IL-3-induced activation of protein kinases in the mast cell/megakaryocyte R6-Xe.4 line.* J. Immunol. *144,* 1759–1766 (1990).

[41] J. P. M. Evans, A. R. Mire-Sluis, A. V. Hoffbrand and R. G. Wickremasinghe, *Binding of G-CSF, GM-CSF, tumor necrosis factor-α and τ-interferon to cell surface receptors on human myeloid leukemia cells triggers rapid tyrosine and serine phosphorylation of a 75-Kd protein.* Blood *75,* 88–95 (1990).

[42] B. Gasllis, K. S. Pricket, J. Jackson, J. Slack, K. Schooley, J. E. Sims, S. K. Dower, *IL-1 induce rapid phosphorylation of the IL-1 receptor.* J. Immunol. *143,* 3235–3240 (1989).

[43] H. L. Goldberg, M. M. Viegas, B. L. Margolis, J. Schlessinger, and L. L. Skorecki, *The tyrosine kinase activity of the epidermal-growth-factor receptor is necessary for phospholipase A_2 activation. Biochem. J. 267,* 461–465 (1990).

[44] B. L. Gruber, L. D. Kaufman, M. J. Marchese, W. Roth, A. P. Kaplan, *Anti-IgE autoantibodies in systemic lupus erythematosus. Prevalence and biologic activity.* Arthritis & Rheumatism *31,* 1000–1006 (1988).