Immunosuppression and the rheumatic diseases

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SUMMARY Ignorance of the basic nature of rheumatoid arthritis precludes the introduction of rational schemes for using cytotoxic drugs. It is still plausible that the autoimmune and other immunological abnormalities which accompany this disease are the secondary effects of persistent antigen, for example, related to microbial infections. In this event, cytotoxic drugs may diminish the inflammatory response but their effects on immune responses would be irrelevant or even undesirable. Should rheumatoid arthritis prove to be a primary immunoproliferative disorder, cytotoxic drugs may prove to be of value not because of their conventional immunosuppressive effects but because of their selective action on the proliferating cells. Indeed, current evidence suggests that these drugs enhance rather than depress conventional immune responses, at least in the doses given to patients with rheumatic disorders.

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Cytotoxic drugs were introduced into rheumatological practice because of rather simplistic views about the pathogenesis of these disorders. The basic abnormality was considered to be a loss of tolerance to self-antigens, allowing the proliferation of abnormal clones of lymphocytes with self-reactivity.²¹⁰ Cytotoxic drugs, it was thought, might eliminate these clones thereby terminating autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. Furthermore, there was experimental evidence that drugs such as cyclophosphamide might aid and abet the re-establishment of tolerance. The situation is now recognised to be far more complicated. There is no longer such confidence that these diseases can be explained by simple unitarian theories, because autoimmune phenomena can be induced by a variety of mechanisms and need not reflect the basic pathogenetic process in these diseases. Moreover, cytotoxic drugs and other immunosuppressive agents do not have a simple dose-related effect on immune responses, a possibility which earlier knowledge of immunological mechanisms could not take into account.

It is now accepted that conventional and autoimmune reactions involve the interactions of several

populations of lymphocytes and accessory cells, both with varying sensitivity to immunosuppressive agents. Above all, there are populations of lymphocytes which control the nature and magnitude of all immune responses whether these are mediated by lymphocytes, antibodies, or even by non-specific 'natural killer' (NK) cells. Thus immunosuppressive drugs, which interfere selectively with the control of immune responses, may increase the vigour of such responses if the cells which effect these responses are themselves resistant to the agent in question.

There are additional difficulties because the causes of most inflammatory connective tissue diseases are unknown. Until recently techniques for assaying immune function in clinical practice have been crude and inexact. Few clinical studies have taken pharmacokinetic factors into consideration when assessing the effects of cytotoxic drugs on immune reactions. It is hardly surprising, therefore, that there should be little reliable information about the crucially important ways in which immunosuppressive agents affect the clinical course of these diseases.

Persistent antigens and connective tissue diseases

Logic, rather than practical results, still inspires the belief that persistent inflammation in the synovial membrane and other tissues affected by rheumatic disorders results from persistent antigen. Quite apart from the obvious interest in efforts to isolate the provoking 'agent', this approach has important implications for interpreting the immunological aberrations which abound in the rheumatic diseases, and thus for the correct line of treatment. If a disease such as rheumatoid arthritis is induced by persistent antigen, the disorder should logically be regarded as a form of immunodeficiency because of the host's inability to eradicate the causative agent. The arthritis and autoimmune features which accompany gross immunodeficiency are often attributable to specific infections. This arouses the suspicion that more specific forms of immunodeficiency to precisely defined organisms might account for similar disorders in patients without evidence of general immunodeficiency.¹⁰

Many of the causes of antigen persistence are also

liable to produce immunological abnormalities. These are of two kinds, the first being depressed immune responses to the antigen itself, and the second being indirect in nature. For example, one postulated source of persistent antigen is bacterial cell wall products which escape immune elimination or enzymatic degradation.¹⁷ Bacterial cell walls, or peptidoglycans isolated from this source, induce a variety of inflammatory lesions in experimental animals, the distribution of which variously resembles rheumatoid arthritis or diffuse inflammatory connective tissue diseases. In addition, bacterial cell wall peptidoglycans stimulate a brisk proliferative response by lymphocytes and this is usually matched by equivalent antibody response.¹¹⁷ Nevertheless, bacterial infections may fail to induce an appropriate antibody response. One notable example is provided by experimentally infecting mice with living or killed cocci.⁸³ The infected mice show a severely impaired immune response against the cell wall antigens in the invading micro-organisms. In addition, the infected mice display a variety of immunological aberrations which closely resemble those observed in patients with rheumatic disorders. These include a diminished capacity to react against other antigens, the production of autoantibodies resembling rheumatoid factor and the generation of antibodies to bacterial cell wall glycoproteins which cross react with lymphocyte membrane glycoproteins. These lymphocyte reactive autoantibodies are analogous to lymphocytotoxins, and show a restricted specificity for those lymphocyte antigens which are displayed only during the non-proliferative phase of the cell cycle. A wide spectrum of autoantibodies is also observed in experimental animals with chronic parasitic infections such as trypanosomiasis.¹¹⁹

Virus infections of man and experimental animals also induce a varying range of immunosuppressive effects and immunological aberrations.⁴³ A number of mechanisms contribute to these effects. In man the most important of these is the ability of viruses to grow in sub-populations of those lymphocytes which are essential for the induction of specific immune responses.¹⁷³ There is little evidence that virus infections in man are potentiated by the ability of the invading virus to inactivate the specific host immune response to the virus in question. However, there are human diseases in which specific immune defects do predispose to an unusually severe or atypical infection. A notable example is the wide range of immunoproliferative disorders induced by Epstein-Barr (EB) virus infections in patients with pre-existing defects in specific cell mediated immunity to this virus, or possibly with defects produced by the infection itself. Thus, immunological aberrations which may result from persistent infection include

impaired immune responses, hypergammaglobulinaemia, rheumatoid factor and auto-antibodies of varying specificity, all of which have often been considered to be characteristic of primary autoimmune disease. This conclusion has prompted the therapeutic assumption that the suppression of such abnormalities will ameliorate the disease. However, if these abnormalities are the result, rather than the cause, of the inflammatory lesions in diseases such as rheumatoid arthritis, there are different therapeutic implications. Obviously, simply correcting these defects would have little relevance to the primary cause of the inflammatory lesion. Equally obviously, the most appropriate way of correcting these abnormalities would be to remove the source of persistent antigen, but this can rarely be achieved. Nevertheless, it is pertinent to this argument that immunological aberrations are observed in patients with sub-acute bacterial endocarditis, including hypergammaglobulinaemia and the production of rheumatoid factor and antinuclear antibodies, but these abnormalities disappear in patients whose disease responds to antibiotics. Similarly, the range of autoimmune phenomena provoked by drugs may equal that observed in patients with systemic lupus erythematosus of unknown cause, and these immunological abnormalities also resolve once the offending drug has been withdrawn.²³⁵

Given that the object of immunosuppressive treatment in most patients with rheumatic diseases is to control the disease process rather than to eliminate the cause, one can question the extent to which such treatment can be expected to provide therapeutic benefit if the immune aberrations are of secondary importance. There are a number of ways in which cytotoxic drugs, and other immunosuppressive agents, can blunt the inflammatory changes induced by antigen persistency. In general terms these involve the depression of inflammatory reactions which are set in train by specific immune events and are too well-known to need further description. These involve humoral mechanisms with detectable circulating immune complexes, and a variety of cellular events mediated by granulocytes, cells of the monocyte-macrophage series, 'natural killer' cells and K cells. Experimental observations in man testify to the suppressive effects of these drugs on inflammatory reactions and, perhaps the most telling observation of all, in the frequency of opportunistic microbial infections and the reactivation of viral infections consequent upon such treatment.

Nevertheless, some fundamental questions remain unanswered. The first concerns the extent to which cytotoxic drugs really reinforce the effects of steroids on these inflammatory events, or whether they have a more fundamental effect on the immunological reac-

tions which induce the inflammation. It is in this context that the complexity of immune responses makes this a difficult question to answer. A variety of suppressor cells have been detected in man that could interfere with efficient responses to persistent antigens. For example, a sub-population of T lymphocytes interferes with natural cytotoxicity against virus-transformed cell lines normally mediated by NK cells.⁴¹ There is good evidence also that various suppressor cell populations are activated in human hosts chronically infected with different microbial infections.²¹⁷ Thus the inability to eliminate the putative persistent agent in a disease such as rheumatoid arthritis could also result from suppressor cells interfering with the generation of an efficient cell-mediated immune response against persistently infected cells. There is, for example, good evidence that delayed hypersensitivity reactions to soluble proteins such as ovalbumin and bovine gammaglobulin are controlled by suppressor T lymphocytes. Cyclophosphamide ablates this suppressor mechanism, thereby producing exaggerated delayed hypersensitivity responses to these antigens.¹⁶⁸ The effects of cytotoxic drugs in connective tissue diseases are, therefore, unpredictable. Logically, these drugs could overcome a defective immune response to a persistent antigen and improve responses to unrelated antigens in addition.

The effects of immunosuppressive agents on immunoproliferative diseases

The clonal theory of autoimmune diseases has been strengthened by recent observations concerning the normal generation of immune responses. It is now apparent that immune responses to foreign antigens are initiated by T lymphocytes which see such antigens in combination with self-antigens. T lymphocytes destroy target cells bearing foreign antigens most efficiently when the target cells and the T lymphocytes concerned in their destruction have histocompatibility antigens in common. Indeed it is now proposed that the principal function of histocompatibility antigens is to expedite the surveillance function of T lymphocytes.¹⁶⁰ Autoimmune reactions, therefore, are no longer viewed as qualitatively different totally abnormal responses, compared with conventional responses, to exogenous antigens; it is the capacity of autoreactive cells to escape normal regulatory mechanisms which determines whether or not such responses will predominate. This concept has been strengthened by repeated observations that autoreactive B lymphocytes are found in normal subjects.²¹⁰ These regulatory defects were initially thought to involve control mechanisms such as suppressor T lymphocytes and the capacity of the normal immune system

to generate antibodies with specificity for unique V region sequences (idiotypes) in the immunoglobulin molecule. More recently it has been established that the generation of antibody diversity in B lymphocytes involves a number of recombination steps between genes coding for different portions of the heavy and light chain molecule. Thus, there is great potential for errors in the sequence of events by which B lymphocytes synthesise antibodies of different isotypes and of different specificities. Consequently, more attention has been given to the possibility that autoreactive B lymphocytes may proliferate in an uncontrolled manner, because of errors in the differentiation of the precursor stem cells for these B cells or in their maturation.⁵⁰

So far, the clonal origin of lymphoproliferative diseases has been established only in malignant forms, such as chronic myeloid leukaemia and polycythaemia vera. To some extent, cytogenetic and immunochemical analysis of both membrane-bound and secreted immunoglobulins will establish this point. However, the point can be made most clearly in black females who are heterozygous for the X-linked enzyme glucose-6-phosphate dehydrogenase and who developed myeloproliferative diseases.⁶⁷ The cellular origin of these disorders can be examined in detail in such subjects. B lymphocytes in diseases such as chronic myeloid leukaemia and polycythaemia vera are derived from a single abnormal malignant clone even though these B lymphocytes produce immunoglobulins with the full repertoire of heavy and light chain determinants, a property previously considered to be characteristic of B cells of polyclonal origin.¹⁴² It has also been clearly established that the autoantibodies in some patients with cold agglutinin disease and haemolytic anaemia are also of clonal origin. There is an attractive possibility, therefore, that the apparently polyclonal antibodies in other common autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, may also be produced by B lymphocytes derived from a limited number of progenitor stem cells. By this concept, rheumatic disorders would be considered as primary lymphoproliferative disorders, and this would explain the failure to find the postulated persistent antigens at sites of chronic inflammation. So far there is no firm evidence of a similar nature in immunoproliferative diseases. Nevertheless, there are many striking similarities between the immunological features of the rheumatic diseases and those detected in murine models of human autoimmune disease.⁵⁰ The immunopathological events in these mice have been intensively studied and the primary defect involves the differentiation and maturation of the B cell progeny of bone marrow stem cells. Moreover NZB mice at

first display a polyclonal hypergammaglobulinaemia, but monoclonal paraproteins subsequently emerge. This process can be correlated with the emergence of a single abnormal clone, detectable cytogenetically, whose progeny eventually infiltrate the spleen and lymphoid system.

There have been many attempts to demonstrate that there is a fundamental abnormality in the regulation of immune responses in patients with rheumatic diseases. Most attention has been directed at demonstrating a defect of suppressor T lymphocytes and abnormalities have been reported in patients with systemic lupus erythematosus and their relatives. Until recently, less attention has been given to seeking defects in B lymphocyte function. However, it is now clear that such abnormalities exist. Cultured B lymphocytes from patients with systemic lupus erythematosus spontaneously synthesise large amounts of immunoglobulin *in vitro*, but are refractory to pokeweed mitogen, a mitogen which normally induces polyclonal immunoglobulin synthesis by human B lymphocytes. Moreover, patients with this disorder show impaired *in vitro* and *in vivo* antibody synthesis in response to immunisation with tetanus toxoid.¹⁵⁹ Techniques are now available for analysing the control of specific antibody synthesis *in vitro*. Recent observations¹⁷² have shown that whereas the lymphocytes from normal subjects produce antibody after *in vitro* challenge with influenza viral antigen, lymphocytes from patients with systemic lupus erythematosus fail to respond in this manner. Experiments in which isolated populations of T and B lymphocytes from lupus patients and from normal controls were co-cultured *in vitro* show that the defect resides in the B lymphocyte population. Thus B lymphocytes from lupus patients were able to function normally as 'helper' cells in specific antibody synthesis, but B lymphocytes from lupus patients were still defective even when combined with 'helper' T cells from normal donors. One can propose that systemic lupus erythematosus is an oligoclonal lymphoproliferative disorder in which B lymphocytes with memory for previous immunological events express some conventional antibody response, but the majority of immunoglobulin synthesis is programmed to produce autoantibodies. Speculation continues about the basic defect which could initiate this uncontrolled proliferation. There are many, admittedly incomplete, hints about the possible nature of these initiating insults. Drugs which bind DNA may induce a syndrome resembling spontaneous systemic lupus erythematosus, and subjects who acetylate such drugs poorly are particularly prone to develop this complication.¹⁶ There is also a clinical impression that intercurrent virus infections exacerbate inflammatory connective disorders. It is there-

fore possible that viruses and drugs act as promutagens, that is, agents which bind to DNA and induce mispairing with other bases.¹²² If not excised by the appropriate excision and repair enzymes, persistence of promutagens could, in proliferating lymphocytes, produce mutations coding for abnormal immunoglobulins with autoreactive specificities. There are indications that lymphocytes from patients with inflammatory connective tissue diseases, including rheumatoid arthritis, are abnormally susceptible to promutagens such as alkylating agents and low-dose ultraviolet irradiation, and this sensitivity appears to be related to a deficiency in removing alkylated bases from lymphocyte DNA.⁸⁸

Should inflammatory connective tissue diseases prove to result from primary immunoproliferative diseases, this would have clear implications for the ways in which immunosuppressive agents might operate. Under these circumstances the aim of treatment would be to eradicate abnormal auto-antibody producing cells in a manner analogous to the treatment of malignant lymphoproliferative diseases. Moreover, drugs such as cyclophosphamide and azathioprine would be more likely to suppress these cells rather than lymphocytes producing conventional antibodies, since such lymphocytes are highly resistant to cytotoxic drugs. Indeed the resistance of conventional secondary antibody responses, but sensitivity of autoantibody production to cytotoxic drugs, points to this conclusion.

Clinical observations in patients receiving immunosuppressive agents

Considerable information has accumulated concerning the effects of immunosuppressive agents on various immunological functions. Unfortunately, the results have been conflicting and confusing. Such criticisms particularly concern attempts to assay the *in vitro* function of lymphocytes isolated from patients receiving immunosuppressive drugs. Technical problems account for some of the discrepancies in published results. Most investigators have carried out tests of lymphocyte function *in vitro* using a single concentration of cultured cells and a standard mitogen challenge. However, it is now clear that the conditions for detecting peak responses to mitogenic stimuli vary in different diseases and are affected by many secondary factors.⁶⁵ Another problem is the understandable failure of earlier workers to appreciate the complexities of immune responses. It is now apparent that meaningful assays of immune function must include tests for all the populations of inflammatory cells which contribute to tissue damage and which modulate these effects. Two principal strategies are being introduced. The first relies on the

ability of monoclonal antibodies to identify suitable markers of each function of sub-populations of lymphocytes, and to score these both in the peripheral blood and in the inflammatory lesions of patients with rheumatic disorders. The second involves functional assays, such as specific antibody production, and, of equal importance, suppressor cell activity. So far there is too little evidence to make dogmatic statements about the extent to which different immunosuppressive agents, or regimens, have selective effects on the different cell populations and immune mechanisms which contribute to the disease process. Nevertheless, there are already firm indications that theoretical immunosuppressive regimens do not predictably suppress conventional immune responses as judged by current standard techniques. Indeed, most observations of standard immune responses in such patients have indicated that these responses were as often increased as depressed by cytotoxic drugs. Paradoxically, too, immunosuppressive treatment commonly improves bone marrow function and host responses to infection in subjects with bone marrow depression and immunodeficiency attributable to connective tissue diseases. This point is emphasised by experience in a comparative trial in which patients with systemic lupus erythematosus, polyarteritis, Behçet's syndrome, or dermatomyositis have been treated with steroids alone in conventional doses, or with a combination of high dose steroid, antilymphocyte globulin and cytotoxic drugs.⁴⁴ Contrary to expectations, bone marrow function and resistance to infection improved more rapidly in patients receiving the more intensive regimen. This point is illustrated by two clinical examples.

The first (fig 1) is a 16-year-old patient with systemic lupus erythematosus, presenting with grand-mal fits and skin lesions, with clinical cutaneous vasculitis, hypertension, hepatosplenomegaly and progressive renal disease. He had strongly positive tests for antinuclear antibody, circulating immune complexes in high titre and hypocomplementaemia. He failed to respond to steroids in high dosage (up to 100 mg prednisone daily) and developed congestive heart failure and staphylococcal septicaemia. Treatment with intensive immunosuppression produced a full clinical remission and the disappearance of most of the characteristic serological abnormalities. However, it is noteworthy that his haemoglobin and white cell count also returned to normal on treatment. Depressed lymphocyte function, which had been detected *in vitro* associated with his disease, also reverted to normal on allegedly immunosuppressive treatment.

The second case is an 18-year-old boy who developed polyarteritis with a mononeuritis multiplex, vasculitis leading to digital ischaemic changes

FIG 1 Bone marrow recovery in a patient with systemic lupus erythematosus receiving intensive immunosuppression. (C3=complement, DNA=DNA binding assay, IC=immune complexes, ANA=antinuclear antibody reciprocal titre.)

FIG 2 Response of polyarteritis to immunosuppressive treatment. The three phases of the disease are described in the text. Cytotoxics: azathioprine and cyclophosphamide; prednisone: 150 mg/day maximum; anti-platelet: aspirin, sulphinpyrazone, tolazoline. ALG=antilymphocyte globulin.

and renal disease (fig 2). The first phase of his illness was characterised by severe clinical disease which responded to immunosuppression with high dose steroids (maximally 150 mg prednisone daily), azathioprine, cyclophosphamide and antilymphocyte globulin (ALG). His disease went into remission and all drugs were withdrawn (phase 2). However, the vasculitic lesions and neuropathy reappeared although, at this stage (phase 3), the lesions were characterised histologically by an obliterative endarteritis rather than by inflammatory

vasculitis. He eventually recovered with further treatment with high dose steroids and cyclophosphamide. It was noteworthy that resistance to secondary infections accompanied periods of active disease but were controlled once adequate immunosuppressive regimens were instituted.

These findings do not help us to distinguish between the two major hypotheses explaining the clinical and immunological abnormalities of the rheumatic diseases. Improved conventional immune responses and the disappearance of immunological anomalies, such as autoantibody production, would be expected if cytotoxic drugs corrected the abnormalities consequent on antigen persistence, for example, in association with persistent infection. Equally, if cytotoxic drugs eliminate clones of autoantibody producing cells which are particularly sensitive to these drugs, the proliferation and maturation of B lymphocytes derived from normal stem cells might be improved, thereby allowing conventional immune responses to recover. The immunodeficiency associated with malignant myeloproliferative diseases, such as the leukaemias, is indeed often reversed as the result of chemotherapy which has been regarded as a favourable prognostic sign.

Conclusions

Despite many years of treatment with cytotoxic drugs, no clear picture has emerged of the ways in which these drugs influence inflammatory connective tissue diseases. The issue is therapeutically important since, if steroids produce their undoubted beneficial effect through immunosuppressive mechanisms, then any drugs which potentiate those effects should be clinically beneficial. Ironically, rapid improvements in our knowledge of the pharmacological events in inflammation have called in question the belief that the therapeutic benefits of steroids owe anything to their effects on lymphocytes and immune function. Indeed it is arguable that the latter are undesirable side effects of steroid treatment. Given our ignorance of the pathogenesis of these diseases and the many plausible ways in which cytotoxic drugs could produce benefit, it is hardly surprising that the issues remain unresolved. The quickest way to resolve the dilemma will be to elucidate the pathogenesis of these diseases. In the meantime, technical improvements in assaying responses in clinical practice at least allow the prospects of pursuing meaningful studies concerning the effects of cytotoxic drugs on immune function.