

An approach to the use of immunosuppressive drugs in nonmalignant diseases

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Immunosuppressive drugs are being given to patients with inflammatory diseases of unknown etiology. The drugs in common use have different biochemical properties that may be responsible for their different immunologic and anti-inflammatory properties. These are reflected in clear-cut distinctions in clinical efficacy. A limited number of controlled trials have suggested that certain immunosuppressive drugs are effective in a few human inflammatory diseases. There have been more negative than positive trials. Furthermore, the drugs increase the risk of infection and may predispose to malignancy. Long-term results are not available; until they are, the drugs would be most profitably administered in the context of controlled long-term trials so that rational approaches to therapy may be possible in the near future. Important medicophilosophical questions are raised by the potential benefit and the potential harm of immunosuppressive drugs in the treatment of nonmalignant diseases. The widespread use of immunosuppressive drugs in the therapy of human diseases of unknown etiology is not warranted at the present time.

For many years corticosteroids were thought of as the wonder drugs for use in the therapy of a variety of diseases of unknown etiology. Subsequently it was found that steroids were not as efficacious as was originally hoped. Furthermore, the serious side effects of prolonged corticosteroid therapy increasingly became a deterrent to their continued promiscuous use. Much of the confusion about corticosteroid therapy of inflammatory diseases of unknown etiology might have been avoided had carefully controlled clinical trials been performed before widespread use. Corticosteroids are rapidly being replaced by new wonder drugs, the cytotoxic and immunosuppressive drugs. There are many enthusiastic uncontrolled reports and several controlled trials that support further investigation of these drugs. The immunological nature of some of the diseases has become more apparent, lending circumstantial support to the use of immunosuppressive drugs. However, there are many negative trials. In addition, the drugs have serious, even life-threatening, side effects. It therefore seems appropriate to review the basis for the use of these drugs in human diseases. This paper will not exhaustively review all studies on the subject, but will rather attempt to highlight the major advances and limitations that characterize the present state of knowledge in the field of commonly used immunosuppressive drugs.

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IMMUNOLOGICAL AND ANTI-INFLAMMATORY EFFECTS

Although our knowledge about the effects of immunosuppressive drugs in man is rapidly accumulating, much of our information and almost all comparative data stem from animal studies. Since species differences may be great, extrapolation to man must be done with caution. Commonly used immunosuppressive drugs include drugs of several different classes with different biochemical actions. Methotrexate, a folic acid antagonist, blocks the conversion of folic and dihydrofolic acids to tetrahydrofolic acid by binding to the enzyme dihydrofolate reductase. Such action blocks the one-carbon transfer involved in the synthesis of thymidyllic acid, thereby blocking DNA synthesis. Azathioprine is rapidly converted to 6-mercaptopurine (6-MP) *in vivo*, and the two drugs will be discussed interchangeably although small differences in therapeutic indices may be important. These drugs act as purine analogues, thereby inhibiting nucleic acid synthesis. Nitrogen mustard derivatives such as chlorambucil and cyclophosphamide interfere with cellular metabolism as alkylating agents. Cyclophosphamide may have additional effects through the ability of some of its metabolites to phosphorylate as well as alkylate.

The exact mechanisms by which these biochemical alterations lead to specific immunosuppressive or anti-inflammatory effects are unknown. Methotrexate and azathioprine act only during the S phase of the cell cycle, during DNA synthesis, whereas the alkylating agents work throughout the cell cycle. This would lead to the prediction that methotrexate and azathioprine would act primarily upon rapidly dividing cells, whereas cyclophosphamide might act on slowly dividing as well as rapidly dividing cells. 6-MP was found to work only during the inductive phase of the immune response, blocking the appearance of large pyroninophilic cells,¹ whereas cyclophosphamide may work through many phases of the immune response.^{2, 3} However, methotrexate seems to block a late step in the immune response, the proliferation of small lymphocytes.⁴

Individual immunosuppressive drugs have been found to suppress immune responses to different degrees. The capacity of different drugs to reduce an antibody response was measured in mice using a relatively low drug dosage, the LD₅ (a dose that will kill 5 per cent of the animals). It was found that the most antibody-suppressive drug was cyclophosphamide and the second was methotrexate.⁵ Purine analogues including 6-MP were less immunosuppressive, and fluorouracil and triethylenemelamine were ineffective. In another study cyclophosphamide suppressed the antibody response of guinea pigs; 6-MP and azathioprine were relatively ineffective.⁶ Even among the same general class of compounds one drug may have much more marked suppressive effects than another. Whereas cyclophosphamide markedly suppressed the immune response to sheep red cells, nitrogen mustard and chlorambucil, also with alkylating properties, were not suppressive.³ Other studies have confirmed the relative efficacy in antibody suppression of cyclophosphamide relative to other alkylating agents.^{5, 7} Cyclophosphamide may be more immunosuppressive because, in addition to alkylating properties, some of its metabolites phosphorylate.²

Since cellular immune functions are primarily mediated by different cells from the antibody-producing cells, it might be expected that different drugs

would be more effective in cellular than in humoral immunity. However, it has been found that cyclophosphamide is also very effective in suppressing cellular immune functions. For example, cyclophosphamide but not 6-MP delayed dinitrochlorobenzene (DNCB) responses.⁸ Cyclophosphamide was found to suppress the tuberculin reaction more completely than azathioprine.⁹ In fact, even when high doses of the active metabolite of azathioprine (6-MP) were given to suppress the tuberculin skin test, the animals' lymphocytes responded normally *in vitro* to tuberculin stimulation.¹⁰ This type of study has suggested that azathioprine and 6-MP may have marked anti-inflammatory properties and lesser immunosuppressive properties. In both rats and guinea pigs nonspecific inflammatory responses measured by skin window or invasion of granulation tissue were suppressed to a greater extent by azathioprine than by cyclophosphamide.⁹ In other studies methotrexate has been found to have potent anti-inflammatory properties.¹¹

The anti-inflammatory effects of 6-MP appear to be related to a reduction in large mononuclear cells both locally¹² and in the circulation.¹³ Neither cyclophosphamide nor methotrexate effectively reduced the local infiltration by mononuclear cells.¹⁴ In these and other studies, the anti-inflammatory effects were not correlated with changes in polymorphonuclear leukocytes.^{11, 12}

Studies of these anti-inflammatory effects in man have shown reduction of mononuclear cell infiltration with both 6-MP and methotrexate therapy.^{15, 16} Immunosuppressive effects of azathioprine in man have been claimed and denied.^{3, 10, 16-18} In studies of patients with rheumatoid arthritis and systemic lupus, azathioprine treatment has led to reduction in antibody formation but not the response of lymphocytes to mitogens.^{19, 20} Cyclophosphamide treatment resulted in both a reduction in antibody formation and a suppression of lymphocyte transformation.¹⁹⁻²¹ Whereas cyclophosphamide produced a profound depletion of circulating lymphocytes in man, azathioprine had little effect upon circulating lymphocytes and a greater effect upon polymorphonuclear leukocytes.²⁰

TOXICITY

Before launching into the recorded experience with these agents, I would like to compare immunosuppressive drugs with others that we use daily in clinical medicine: penicillin and aspirin. Penicillin kills the etiological agent of pneumococcal pneumonia. Its value has been obvious without controlled trials. Aspirin is clearly beneficial with rather little toxicity in a variety of conditions, especially rheumatic diseases. Although aspirin is only suppressive rather than curative, its value lies primarily in its relative freedom from toxicity. In contrast, immunosuppressive drugs lack both desirable characteristics: they are not curative and carry major toxic side effects.

The several immunosuppressive drugs share certain toxic properties. The most common is predisposition to ordinary bacterial infection. Such predisposition tends to be greatest in the most severely ill patients—those most likely to be given immunosuppressive drugs. Certain exotic infections also occur too frequently during the course of immunosuppressive therapy. These include infec-

tions with such diverse organisms as cytomegalovirus, *Pneumocystis carinii*, Cryptococci, Candida, and Mycobacteria. Increased fetal wastage or abnormality and the passage of mutations to future generations are unproved but likely possibilities. Immunosuppressive drugs may predispose to malignancy in the future. Although this is clear-cut following organ transplantation,²² it is still theoretical in other situations. Sterilization is a threat with any cytotoxic drug, but is common only with alkylating agents. Other specific problems include hepatic toxicity with ultimate fibrosis secondary to methotrexate, idiosyncratic reaction to azathioprine, and both cystitis and alopecia after cyclophosphamide. Xanthine oxidase inhibitors such as allopurinol cannot be given with azathioprine or 6-MP without drastically reducing the immunosuppressive drug dose since the metabolism of the 6-MP is inhibited. Other purine analogues that are metabolized by other pathways, such as 6-thioguanine, do not share this characteristic. Doses of methotrexate and perhaps other agents should be reduced in renal failure.

CLINICAL STUDIES

Because of the noncurative and toxic characteristics of immunosuppressive drugs in the treatment of nonmalignant diseases, attention must be given to methods of evaluation of efficacy. In many cases, physicians adequately decide whether or not a drug is effective in a certain disorder by personal experience. However, if a drug has marginal benefit and substantial toxicity, personal experience will usually be inadequate. Another method of drug evaluation is through uncontrolled trials. Reports of such trials tend to be weighted in favor of either positive or negative results. Treatment of a series of patients with equivocal results rarely leads to publication. It has therefore become apparent that controlled trials represent the only reliable method of obtaining information regarding the efficacy of immunosuppressive drugs in most nonmalignant diseases. A controlled trial is one in which patients meet the criteria for the study and, consent for the study being obtained, are then assigned randomly to different treatment groups.

More than thirty such controlled trials have been performed in at least a dozen inflammatory diseases of unknown etiology. There are certain diseases in which there is adequate short-term information to indicate the usefulness of immunosuppressive drugs. In certain instances uncontrolled studies may be persuasive. For example, full-blown Wegener's granulomatosis, a rapidly fatal disease when untreated, has been suppressed by azathioprine, methotrexate, and cyclophosphamide. The greatest experience is with cyclophosphamide and is impressive.^{23, 24} However, milder forms of Wegener's granulomatosis may become more easily recognized and diagnosed. Therefore, a controlled trial may be necessary to support the efficacy of toxic drugs in a no longer rapidly fatal form of the disease.

The use of methotrexate in severe psoriasis is supported by a well-done but short-term controlled trial,²⁵ and the disease is now a recognized indication for the drug. It is obvious that only the most severe cases should be so treated since the hepatotoxic effects of methotrexate are significant. After 3 or 4 years of

treatment with methotrexate, many patients developed liver fibrosis and cirrhosis.²⁶ Like psoriasis, psoriatic arthritis has been treated successfully with methotrexate.²⁷ In a controlled trial involving a small number of patients, azathioprine also was found effective in psoriatic arthritis.²⁸

Controlled study has demonstrated the therapeutic benefits of cyclophosphamide in nephrosis,²⁹ whereas azathioprine has not been effective.^{30, 31} Since childhood nephrosis is usually a self-limited disease and cyclophosphamide therapy may alter the reproductive capacity in addition to its other side effects, the use of cyclophosphamide in childhood nephrosis is being re-examined.

Negative results have been found for the use of azathioprine in iridocyclitis,³² nephritis,^{33, 34} Crohn's disease,³⁵ chronic active hepatitis,³⁶ asthma,^{37, 38} and multiple sclerosis (Zweiman, personal communication). Azathioprine was of questionable value in biliary cirrhosis.³⁹ Cyclophosphamide has been ineffective in multiple sclerosis.⁴⁰ It was ineffective in combination with azathioprine in nephritis³³; however, an uncontrolled study has suggested that the combination of azathioprine plus cyclophosphamide plus steroids may be effective in nephritis.⁴¹

The largest number of controlled trials have been performed in two diseases: rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). I will discuss these in detail to indicate the basis for use and the problems involved. The first question raised was the goal of therapy. When immunosuppressive drugs were first introduced it was hoped that they might be curative. It has become clear that this is an unrealistic expectation. The drugs may be suppressive but cannot regularly effect a cure. Furthermore, Fries and his co-workers⁴² found that cyclophosphamide could not be used as the primary drug in diseases such as SLE and polymyositis. Particularly in SLE it appears that steroids are frequently necessary to suppress a variety of systemic symptoms. Furthermore, as the toxicity of the immunosuppressive drugs is becoming better understood, it seems likely that these agents will not be used as primary drugs in diseases such as RA since much less toxic medicines are available for primary therapy. Immunosuppressive drugs may allow the reduction of corticosteroid dosages; both azathioprine and cyclophosphamide appear to satisfy this goal. In an example from Great Britain,⁴³ patients with severe RA requiring prednisone therapy were randomized to receive either a placebo or azathioprine and they were then followed for 12 months. There was a constant attempt to reduce the steroid dose without increasing disease activity. At the end of the year it was found that the group who had received azathioprine had a significant reduction in steroid requirement as compared with the placebo group.

Other studies have added immunosuppressive drugs to a constant dose of other drugs to determine whether or not immunosuppressive drug or placebo was more effective. High doses (about 1.5 to 3.0 mg. per kilogram per day) of either azathioprine or cyclophosphamide have been effective in controlled trials in the therapy of RA.^{28, 44-46} However, it appears that the efficacy of cyclophosphamide in RA is dose-dependent. Given in doses between 0.7 and 1.4 mg. per kilogram per day, cyclophosphamide is no better than placebo.^{47, 48} In addition, the toxic side effects of the drug were almost as great at the low dose.⁴⁸

In summary, the controlled trials of azathioprine and cyclophosphamide in the therapy of severe RA suggest that both drugs, when given in sufficient quantity, reduce disease signs and symptoms. However, toxic effects of these drugs have also been observed. This raises an important medical-philosophic question: "Is it the physician's job to decide between the quality and quantity of life?" With the potential use of immunosuppressive drugs in the treatment of RA and other diseases, we may in fact, as physicians, have the ability to improve the quality of life and yet reduce the quantity of life, at least on a statistical basis.

Let us turn now to the therapy of systemic lupus erythematosus. In the original NIH study, cyclophosphamide was compared with placebo for 10 weeks in-hospital in a double-blind trial.⁴⁹ This study suggested that cyclophosphamide was useful in the therapy of lupus nephritis. However, the study was short-term with a relatively small number of patients.

In a controlled trial of SLE not restricted to patients with nephritis, azathioprine led to increased creatinine clearance and decreased proteinuria.⁵⁰ Furthermore, patients receiving azathioprine required significantly less prednisone. In a study of SLE nephritis, an average dose of prednisone (40 mg. per day) was compared with that dose of prednisone plus azathioprine (2 to 3 mg. per kilogram per day). After 6 months of therapy it was concluded that no benefit was obtained by the addition of azathioprine to therapy with prednisone.⁵¹ At the NIH we have been comparing the use of cyclophosphamide versus azathioprine versus placebo in the therapy of lupus nephritis.²⁰ In a 10 week period, the urine sediment has improved primarily in the cyclophosphamide group. Serum complement has also been increased more by cyclophosphamide than by azathioprine. Anti-DNA antibodies have been improved equally by cyclophosphamide and azathioprine. Overall, patients receiving cyclophosphamide have done significantly better than both the azathioprine- and the placebo-treated groups. Cyclophosphamide has also been superior in the renal measures. Azathioprine has been statistically better than placebo overall, but not with regard to the renal measures. Our patients with SLE receiving either azathioprine or cyclophosphamide have had severe infections with such organisms as *Pneumocystis carinii*, cytomegalovirus, and *Torulopsis glabrata* in addition to miliary tuberculosis, *Pseudomonas* abscesses, pneumococcal peritonitis, and bacterial pneumonia. In a limited long-term follow-up there appears to be no more toxicity with cyclophosphamide than with azathioprine. The controlled information in SLE suggests that both azathioprine and cyclophosphamide are potentially effective adjuncts to therapy and that cyclophosphamide may be superior, but that more long-term information is necessary to determine whether the beneficial effects outweigh the toxic ones.

ANIMAL STUDIES

Therapeutic studies in NZB/W F₁ mice suggest that immunosuppressive drugs may be used to greater advantage by altering treatment programs. The lupus-like syndrome of these mice responds to either steroids or azathioprine or cyclophosphamide. Certain combinations of two drugs may be superior to

single-drug therapy.^{52, 53} All three drugs together have been even more effective without increased toxicity.⁵⁴

Another method of administration that is being investigated is intermittent high-dose therapy instead of daily low-dose therapy. Preliminary experiments suggest that the same drug dose may be more effective if given intermittently.⁵⁵

Specific tolerance⁵⁶ elimination of a particular class of antibody-forming cells^{3, 57} and the administration of an antimetabolite followed by the metabolite⁵⁸ may lead to more specific and less toxic use of immunosuppressive drugs in therapy of nonmalignant diseases.

CONCLUSION

As physicians we are obliged both to relieve suffering and to do no harm. It may not be possible to achieve both when caring for patients with inflammatory diseases of unknown etiology. If a physician withholds immunosuppressive drugs he may fail to relieve suffering. If he gives immunosuppressive drugs he may induce a life-threatening infection. This conflict is of both philosophic and practical importance. It is only partially solved by guidelines for the use of cytotoxic drugs in human nonmalignant diseases.⁵⁹

Since the drugs are not curative, and may be quite toxic, their use should be supported by carefully performed controlled trials. Patient selection should lead to treatment of patients not doing well on standard therapy even in those diseases in which immunosuppressive therapy has been supported by short-term controlled trials, e.g., psoriasis, nephrosis, rheumatoid arthritis, and systemic lupus erythematosus. Since there are no long-term trials in any disease, it is desirable that such treatment take place in the context of a long-term controlled trial so that we may gain the information required to make rational decisions. Until these are available, caution should be exercised in the use of immunosuppressive drugs for inflammatory diseases of unknown etiology. Finally, we should continue to search for the causes of these diseases with the hope that such information will lead to more specific therapy.

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