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IMMUNOSUPPRESSIVE THERAPY*

The Relation between Clinical Response and Immunologic Competence

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SINCE the demonstrations that 6-mercaptopurine inhibits antibody synthesis[1] and allograft rejection[2] in laboratory animals, this inhibitor of purine metabolism and its analogue, azathioprine, have been used for the treatment of immunologic diseases and the prevention of organ graft rejection in man with increasing frequency.[3,4] More recently, a folic acid antagonist, amethopterin, has also been advocated for the treatment of immunologic diseases.[5] Despite many investigations, there is still considerable uncertainty regarding the therapeutic indications, side effects and mechanisms of action of these agents. In the present study, the answers to the following questions were sought: What is the extent of immunosuppression induced by antimetabolite therapy in patients with immunologic diseases? Is drug-induced immunosuppression harmful to the patient? Is the induction of bone-marrow depression necessary for immunosuppression? Is there a correlation between immunosuppression and clinical results? Can measurement of a defined immune response be used as a guide to therapy? The information presented below was obtained by tests of immune responses in 20 patients undergoing treatment with azathioprine or amethopterin for diseases assumed to have an immunologic basis.

MATERIALS AND METHODS

Clinical Material

The patients are listed in Table 1. In every case corticosteroid treatment either had failed to affect the disease or had produced such undesirable effects as psychosis, osteoporosis or peptic ulceration. Two antimetabolites, azathioprine§ and amethopterin, were used. The latter drug, excreted mainly in urine and bile, was considered contraindicated in patients with impaired kidney or liver function. Azathioprine was given by mouth, 3.0 mg. per kilogram of body weight per day. Amethopterin¶ was given intravenously, 50 to 75 mg. to each patient every five to seven days. In all cases the minimum duration of therapy was six weeks. When antimetabolite treatment was instituted all patients had active, chronic, unremitting disease. The progress of the disorder was evaluated by as many objective measures as were available for the disease under study.||

Immunologic Methods

The primary immune response was evaluated after immunization with keyhole-limpet *(Megathura crenulata)* hemocyanin (KLH). This antigen, obtained from the Pacific Biomarine Company, Venice, California, was purified according to the method of Weigle.[6] The final preparation was *associated* KLH, the high-molecular-weight form of this substance. Sodium colistimethate (Coly-Mycin), 500 γ per milliliter, and oxacillin, 1000 γ per milliliter, were added to the stock solution of hemocyanin (25 mg. per milliliter) to ensure sterility. All patients were given 5 mg. of KLH subcutaneously on the first day of antimetabolite therapy. The circulating-antibody responses of the patients were compared to those of 10 normal controls given the same amount of KLH. We assume that the antigen elicits a primary immune response in man since: antibody to KLH was not found in the serums of 50 persons not immunized with this antigen; to the best of our knowledge, none of our subjects had ever been exposed

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TABLE 1. *Original Data on the 20 Patients in This Study.*

PATIENT	AGE yr.	SEX	DIAGNOSIS	ANTIMETABOLITE
P.C.*	23	F	Systemic lupus erythematosus	Amethopterin
M.M.*	33	F	Systemic lupus erythematosus	Amethopterin
C.L.*	38	F	Systemic lupus erythematosus	Amethopterin
D.S.*	47	M	Ulcerative colitis	Azathioprine
E.F.†	30	M	Ulcerative colitis	Azathioprine
A.H.†	25	F	Ulcerative colitis	Azathioprine
R.M.*	15	M	Ulcerative colitis	Azathioprine
J.M.*	63	M	Autoimmune hemolytic anemia	Azathioprine
R.L.*	54	F	Autoimmune hemolytic anemia	Azathioprine
H.B.*	34	F	Idiopathic thrombocytopenic purpura	Azathioprine
R.C.*	46	F	Idiopathic thrombocytopenic purpura (systemic lupus erythematosus)	Azathioprine
J.Mc.†	55	M	Nephrotic syndrome	Azathioprine
T.S.†	18	M	Nephrotic syndrome	Azathioprine
G.Z.†	36	F	Lupus nephritis	Azathioprine
R.K.†	18	M	Nephrotic syndrome	Azathioprine
T.H.†	53	M	Nephrotic syndrome	Azathioprine
S.P.†	22	M	Nephrotic syndrome	Azathioprine
J.D.†	22	F	Vasculitis	Azathioprine
D.G.*	47	M	Polyneuritis	Amethopterin
G.L.*	17	M	Polymyositis	Amethopterin

*Corticosteroids withdrawn upon institution of antimetabolite treatment.

†Corticosteroids not given for at least 1 mo. before beginning of antimetabolite treatment.

to this material, an extract of the hemolymph of an inedible mollusk inhabiting the coastal waters of Southern California; and the character of the immune response in normal subjects was that of a typical primary response.

We tested the secondary immune response by measuring antibody levels after immunization with diphtheria toxoid. This material, obtained from the Massachusetts Department of Public Health, Biological Laboratories, was administered (0.5 ml., subcutaneously) without further processing.

Serum antibodies were estimated by the tanned red-cell technic.[7] Two classes of antibodies were distinguished on the basis of their sensitivity to 0.1 molar 2-mercaptoethanol (2-ME).[8] The sensitive antibodies were assumed to be γM antibodies and the resistant antibodies were assumed to be γG immunoglobulins. In a limited number of cases, density-gradient ultracentrifugation of the serum showed that the 2-ME-sensitive antibodies were heavy, presumably IgM molecules, and that the 2-ME-resistant antibodies were light, presumably IgG molecules. The possible participation of other immunoglobulins (IgA and IgD) was not, however, ascertained or ruled out.

Delayed hypersensitivity was tested by observation of the local reaction to an intracutaneous injection of 100 μg. of KLH given five or more days after the subcutaneous dose of that antigen. In many cases these skin tests were repeated at monthly intervals. KLH was found to produce a typical tuberculin-type reaction in each of 10 normal persons within three to five days after the primary immunization. In all cases the reactions were considered positive if an erythematous, indurated area measuring at least 5 mm. in diameter appeared at the site of the skin test within twenty-four to seventy-two hours.

Serum levels of IgG, IgA, and IgM were determined with radial immunodiffusion plates.* All tests were carried out with the same lot of plates to minimize possible variations.

Isoagglutinin titers were measured by a standard saline-agglutinin method.[9]

RESULTS

None of the 20 patients had a normal primary immune response (Table 2). The induction time was normal in only 2; in 2 others it was moderately prolonged (two or three weeks); in 9 it exceeded one month, and in 7, antibody formation was never observed. All 13 patients eventually forming anti-KLH antibodies were receiving antimetabolites at the time of onset of antibody synthesis. In each of these patients the phase of formation of 2-ME-sensitive (IgM) antibody was markedly prolonged. In the 10 normal subjects 2-ME-sensitive antibodies reached peak titers within two weeks and disappeared from the serum by the fifth week after immunization (Fig. 1). At that time, all the circulating anti-KLH antibodies were 2-ME resistant (IgG).† By contrast, of the drug-treated patients who did produce anti-KLH antibodies, IgG antibodies never appeared in 5 (Fig. 1), and in the remaining 8, they were formed only after considerable delays.

Two patients receiving amethopterin elaborated abnormally high titers of anti-KLH antibodies. In G.L. (Fig. 2), the isoagglutinin (anti-B) titer rose spontaneously at the same time. P.C. formed high titers of anti-KLH antibodies within a few days after

*Immunoplates, Hyland Laboratories, Los Angeles, California.

†For simplicity, 2-ME-sensitive antibodies will be considered as IgM, and 2-ME-resistant antibodies as IgG. It is recognized that some IgA molecules may be partially resistant to 2-ME[10] and that the distinction between IgM and IgG on the basis of sensitivity to 2-ME is not always absolute.

TABLE 2. *Primary Immune Response to KLH in 20 Patients Treated with Antimetabolites.*

PATIENT	INDUCTION TIME*	PEAK TITER		SEQUENCE†	DELAYED HYPERSENSITIVITY‡
	days	*maximum $\log_2$ titer*	*days after immunization when titer found*	*days*	*days*
P.C.	6	15	6	6	198
M.M.	46	8	74	§	8
C.L.	§	§		§	§
D.S.	42	9	91	81	§
E.F.	65	4	72	§	9
A.H.	14	8	35	§	§
R.M.	7	9	42	§	§
J.M.	§	§		§	§
R.L.	§	§		§	§
H.B.	§	§		§	§
R.C.	§	§		§	186
J.Mc.	140	10	168	181	83
T.S.	19	12	129	129	188
G.Z.	179	8	179	179	7
R.K.	31	8	51	89	33
T.H.	41	6	103	§	7
S.P.	§	§		§	§
J.D.	§	§		§	§
D.G.	67	9	184	142	130
G.L.	75	16	78	75	7
Normal values¶	7	8.7	16.8	31.5	5

*Before antibody first detected.
†Days before 2-ME-resistant antibodies first detected.
‡Days before hypersensitivity to KLH first detected.
§Not detected for duration of study.
¶Mean values for 10 normal persons.

amethopterin treatment was started. Both patients had excellent clinical responses to the antimetabolite.

In the 19 cases in which it was measured, the secondary response (Table 3) was normal in only 3. In 6 the induction period was greatly prolonged, and in 9 a secondary response was not elicited.

Delayed hypersensitivity to KLH was found in each of 10 normal subjects immunized with that antigen. It was normal in only 5 of 20 patients treated with antimetabolites (Table 3). In 9 cases delayed hypersensitivity was never expressed, and

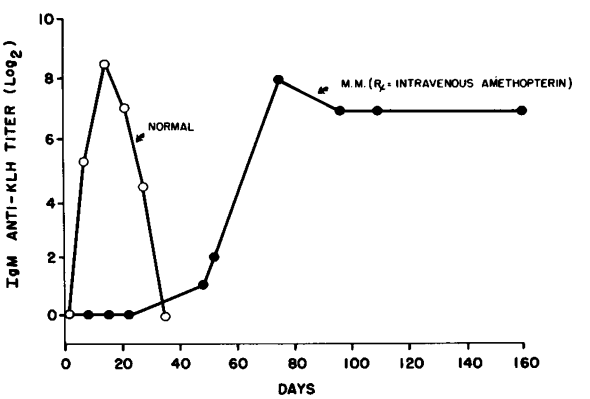

FIGURE 1. *Prolonged Synthesis of IgM Antibodies in a Patient Treated with Amethopterin.*

The normal IgM response to KLH is indicated by o---o---o. The patient had no detectable IgG anti-KLH antibodies for the duration of the study. Note the prolonged induction time.

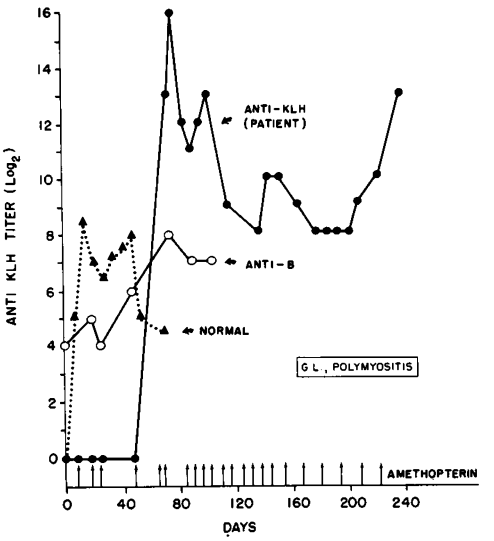

FIGURE 2. *Enhancement of Antibody Synthesis in a Patient Treated with Amethopterin.*

Each vertical arrow represents the intravenous administration of the drug. The immune response of normal persons to KLH is shown by oooΔooooΔooo. Note the spontaneous rise in isoagglutinin titer (anti-B) occurring at the same time as the rise in anti-KLH titers.

in 6, it finally appeared after repeated testing (in 1 patient, after a hundred and ninety-eight days).

The isoagglutinin titers were affected in only 2 cases. In 1 (R.C.) they were reduced, and in the other (G.L.) they were increased.

The IgG levels decreased by 20 per cent or more in 8 of 20 patients; they increased in 2 patients with the nephrotic syndrome, presumably because of a diminution in proteinuria. Decreases in IgA and IgM levels were found in 6 of 18 and 7 of 18 patients, respectively. Rises in IgM concentrations were observed in 2 other patients with nephrosis.

In no patient did antimetabolite treatment result in either anemia or thrombocytopenia. In none of the patients given amethopterin did leukopenia develop. Eight patients treated with azathioprine showed leukopenia, which was usually mild and easily reversed by adjustment of drug dosage. The establishment of leukopenia was not necessary for immunosuppression, since the immune responses of 12 patients with normal white-cell and differential counts were profoundly modified.

Infections developed in 3 of 20 patients. In 2 the disease was diagnosed as viral pneumonia although an etiologic agent was never isolated. These patients, in whom the primary immune response was completely suppressed, made a normal recovery from their infectious disease. One patient died of septicemia due to a gram-negative organism. She had very severe autoimmune hemolytic anemia. Her immune responses, both primary and secondary, were suppressed. The white-cell count, however, was normal up to the time of death.

A comparison between the results of the immunologic tests and the clinical effectiveness of the

TABLE 3. *Immunoglobulin Levels, Isoagglutinin Titers (Log_2) and Secondary Immune Responses to Diphtheria Toxoid in 20 Patients Treated with Antimetabolites.*

PATIENT	IgG		IgA		IgM		ANTI-A		ANTI-B		DIPHTHERIA TOXOID		
	BEFORE TREATMENT	AFTER TREATMENT	BEFORE TREATMENT	AFTER TREATMENT	BEFORE TREATMENT	AFTER TREATMENT	BEFORE TREATMENT	AFTER TREATMENT	BEFORE TREATMENT	AFTER TREATMENT	INDUCTION TIME*	PEAK TITER	INTERVAL BEFORE PEAK TITER FOUND
	mg./100 ml.	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*mg./100 ml.*	*days*		*days*
P.C.	10.5	12.4	2.5	2.4	1.0	1.0					†	†	
M.M.	16.0	16.0	1.9	1.6	1.6	1.1	2	2			†	†	
C.L.	9.4	2.7	2.8	1.8	1.0	0.3			2	2	†	†	
D.S.	17.1	17.0	—	—	—	—	5	5	5	6	†	†	
E.F.	12.0	12.0	3.0	1.4	1.6	0.5					†	†	
A.H.	18.0	10.5	4.1	2.6	1.6	1.8	6	6	5	5	7	9	
R.M.	18.0	14.0	4.8	2.2	7.5	2.7			2	1	†	†	
J.M.	31.0	21.0	1.3	0.7	3.2	2.8					19	†	
R.L.	7.3	6.4	1.9	1.9	0.9	0.7					7	3	16
H.B.	7.2	7.2	1.0	1.0	1.6	1.6	4	3	3	1	19	3	19
R.C.	31.0	24.0	0.2	0.2	6.6	6.6	7	5	10	6	7	7	10
J.Mc.	9.8	11.0	1.5	2.4	0.3	0.4	3	3	3	2	‡	‡	
T.S.	14.5	12.5	1.8	1.5	1.0	0.4					†	†	
G.Z.	21.0	18.5	1.8	1.7	0.5	0.8					24	1	24
R.K.	6.4	4.7	1.0	1.0	1.0	1.1			8	7	9	10	9
T.H.	3.1	7.2	2.2	2.2	0.8	1.6					†	†	
S.P.	27.0	14.0	0.6	0.6	7.5	7.5	5	5	3	4	21	2	21
J.D.	16.0	7.2	4.8	1.8	0.3	0.5			4	4	†	†	
D.G.	7.7	7.0	2.2	2.0	2.0	1.0	8	7	4	3	67	2	75
G.L.	25.1	23.5	—	—					4	8	96	10	109

*Before antibody first detected.

†Not detected for duration of study.

‡Not done.

antimetabolites is shown in Table 4. Five patients entered a complete remission. In 4 of these, circulating-antibody responses were either suppressed or modified. One patient had an enhanced response to KLH. Delayed hypersensitivity was suppressed in 3 and normal in 2. Immunoglobulin levels were suppressed in 2 of 5. Isoagglutinin titers were increased in 1 patient and unaffected in 4.

Six patients had partial remission. The humoral-antibody responses were suppressed or modified in 5 and enhanced in 1. Delayed hypersensitivity was suppressed in all. Immunoglobulin levels and isoagglutinin titers were suppressed in 2 of 6 and 1 of 6, respectively.

Nine patients failed to respond clinically. Circulating-antibody responses were suppressed or modified in every patient except A.H. and R.K., who had normal secondary responses. Delayed hypersensitivity was normal in 4 of 9. Immunoglobulin levels were suppressed in 4 of 9. In no case were isoagglutinin titers affected.

No correlation between the presence, absence or extent of immunosuppression and clinical responses could be found. The results in D.G. suggest that continuous immunosuppression is not necessary to maintain a clinical remission since he proceeded to elaborate anti-KLH antibodies after the discontinuation of amethopterin and prednisone therapy and yet remained clinically well. The almost identical immune responses of 2 patients (Fig. 3) with ulcerative colitis — 1 failing to improve and the other entering a remission — illustrate the lack of correlation between immunosuppression and clinical effect encountered in this series of patients.

DISCUSSION

These studies were substantially aided by the versatility of KLH, a potent antigen long used in animal experiments[11] but never before applied to the study of immunity in man. In our experience, based on studies in over 50 patients, this substance is apparently innocuous. No reactions other than minor, localized pruritic swelling at the site of injection have yet developed. Immunologic cross-reactivity with hemocyanins derived from Atlantic water mollusks does not exist,[12] and we have not encountered in persons sensitized to KLH any examples of allergic reactions on the ingestion of shellfish or other seafood.

Several previous reports[13-17] have demonstrated inhibition of immune responses in man by various antimetabolites. The significance of these results for the treatment of immunologic diseases can only be surmised, since, for the most part, they were obtained in patients undergoing chemotherapy for cancer. In the present investigations, the immunologic responsiveness of patients treated with these compounds for non-neoplastic diseases was assessed and compared with the clinical outcome in an attempt to answer the specific questions mentioned at the beginning of this article. Each of these will now be discussed in light of the data obtained.

TABLE 4. *Comparison between Immunologic Competence and Clinical Results.*

PATIENT	PRIMARY RESPONSE*	SECONDARY RESPONSE*	DELAYED HYPERSENSITIVITY†	IMMUNOGLOBULIN LEVEL‡	ISOAGGLUTININS§	CLINICAL RESULT¶
P.C.	Enhanced	Suppressed	Suppressed	Normal	Normal	Partial remission
M.M.	Modified	Suppressed	Normal	Normal	Normal	Complete remission
C.L.	Suppressed	Suppressed	Suppressed	Suppressed	Normal	Partial remission
D.S.	Modified	Suppressed	Suppressed	Normal	Normal	Complete remission
E.F.	Modified	Suppressed	Normal	Normal	Normal	Complete remission
A.H.	Modified	Normal	Suppressed	Suppressed	Normal	Failure
R.M.	Modified	Suppressed	Suppressed	Suppressed	Normal	Partial remission
J.M.	Suppressed	Modified	Suppressed	Suppressed	Normal	Complete remission
R.L.	Suppressed	Modified	Suppressed	Normal	Normal	Failure
H.B.	Suppressed	Modified	Suppressed	Normal	Normal	Partial remission
R.C.	Suppressed	Modified	Suppressed	Suppressed	Suppressed	Partial remission
J.Mc.	Modified	–	Suppressed	Normal	Normal	Failure
T.S.	Modified	Suppressed	Suppressed	Normal	Normal	Failure
G.Z.	Modified	Modified	Normal	Normal	Normal	Failure
R.K.	Modified	Normal	Normal	Suppressed	Normal	Failure
T.H.	Modified	Suppressed	Normal	Normal	Normal	Failure
S.P.	Suppressed	Modified	Normal	Suppressed	Normal	Failure
J.D.	Suppressed	Suppressed	Suppressed	Suppressed	Normal	Failure
D.G.	Modified	Modified	Suppressed	Normal	Normal	Partial remission
G.L.	Enhanced	Modified	Normal	Normal	Enhanced	Complete remission

*Suppressed, no antibody detected at any time time; modified, no IgG antibody formed for at least 60 days; enhanced, peak titer at least 2 dilution tubes that of any normal subject.

†Suppressed, no reaction to KLH for at least 90 days.

‡Suppressed, at least 20% reduction either in IgG alone or in both IgA and IgM.

§Suppressed, reduction of at least 2 serial dilutions in titer; enhanced (see text).

¶Complete remission, disappearance of all clinical and laboratory evidence of disease; partial remission, subsidence of all major clinical and laboratory evidence of disease; failure, persistence of 1 or more major signs of disease.

What is the extent of immunosuppression induced by antimetabolite therapy? The primary immune response to KLH was affected in every patient. Apart from its complete inhibition, 3 types of abnormalities were found: prolongation of the induction time; delayed or absent synthesis of γG antibodies (even though γM antibodies were formed in normal amounts); and enhancement of antibody synthesis. Each of these effects has been noted in laboratory animals treated with antimetabolites.[18-21]

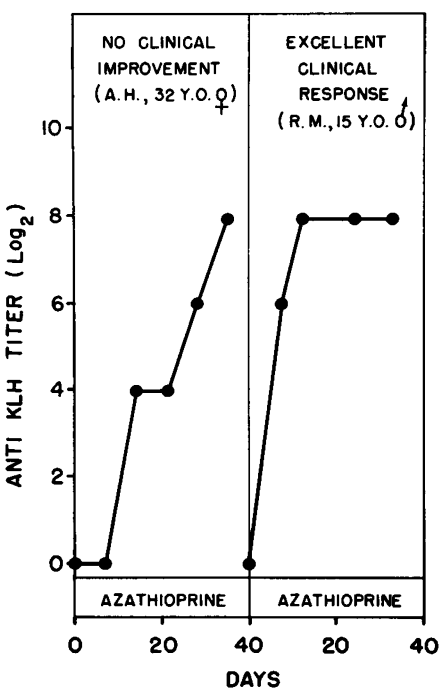

FIGURE 3. *Responses to KLH in 2 Patients with Ulcerative Colitis Treated with Azathioprine.*

In both cases the antibodies were entirely γM. A.H. had a milder example of the disease.

The selective resistance of γM-antibody synthesis to antimetabolites may be of some practical and theoretical interest. For example, disorders due to γM antibodies may be resistant to therapy with the presently available drugs. Cold-agglutinin disease, a condition associated with γM anti-red-cell autoantibodies, is a case in point since in our experience it has been refractory to purine analogues. The contrasting effects of antimetabolites on IgM and IgG formation were revealed in another manner in 2 patients (J.M. and M.M.) whose γG red-cell autoantibody disappeared while they were synthesizing normal amounts of γM anti-KLH antibodies. This phenomenon suggests further possibilities – for example, the selective suppression of reaginic antibodies with a chemical agent. An additional practical implication of this selective immunosuppressive effect is reduction of the risk of infection, since it may not be necessary to inhibit all immune responses to obtain a therapeutic response.

The very prolonged phase of γM anti-KLH synthesis in the drug-treated patients is analogous to findings in 6-mercaptopurine-treated rabbits.[18,19] It suggests that in man, IgG antibodies may be of importance in terminating IgM synthesis. A feedback control of IgM synthesis by IgG has been proposed on the basis of animal experiments showing the action of γG antibodies in stopping the production of γM antibodies.[22-24]

Enhancement of primary antibody responses was observed in 3 patients. A similar, paradoxical stimulation of antibody synthesis by cytotoxic agents has been found in experimental animals.[21,25-28] Its mechanism is obscure, and several explanations have been advanced.[21,29,30] The possibility that the underlying process somehow accentuated the immune responsiveness of these 3 patients should also be considered.

The suppression of delayed hypersensitivity by azathioprine and amethopterin in 15 of 20 patients is again consistent with previous observations in laboratory animals.[31,32] At least in some patients, it is unlikely that this was an anti-inflammatory effect, because in 6, delayed hypersensitivity finally appeared after a prolonged period of chemotherapy. Page et al.[33] have shown that the anti-inflammatory effects of 6-mercaptopurine increase with the duration of chemotherapy.

Reduction in immunoglobulin levels has not regularly been noted in human beings treated with cytotoxic drugs. Wolff and Goodman[34,35] reported hypogammaglobulinemic levels produced in man and rabbits by purine antagonists. McKelvey and Carbone[36] noted decreases in IgG levels in patients receiving intensive chemotherapy for neoplastic diseases. We observed this phenomenon in only 1 patient; in 7 cases abnormally high levels of gamma globulin returned to normal, presumably because the disease had subsided. In agreement with others,[37] we found no constant effect of chemotherapy on isoagglutinin titers.

Is drug-induced immunosuppression harmful to the patient? Immunologic incompetence is associated with a readily definable risk, infection and a potential, but presently unestablished possibility – neoplasia. There is no evidence that drug-induced immunosuppression is associated with an increased occurrence of spontaneous malignant tumors in man. Nevertheless, this possible danger will require careful study over many years.

Invasion by infectious agents is a more immediate and clear-cut threat in the immunologically incompetent patient. Despite this, infection occurred in only 3 of our patients. Two were affected by viral pneumonia and had no unusual complications related to the infection. The third patient died of septicemia caused by a gram-negative organism. Although the role of immunosuppression in potentiating this infection cannot be denied, this woman was gravely ill with severe hemolytic anemia. The low incidence of infections in patients of this type (of the last 50 patients with immunologic diseases treated with antimetabolites in our hospital, only 1 – R.L. – had a lethal infection) is an interesting contrast with 2 other conditions characterized by immunologic incompetence in which serious infections occur with high frequency: agammaglobulinemia[38] and receipt of a kidney graft.[39,40] Patients with immunologic diseases do not become agammaglobulinemic as a result of antimetabolite therapy. Although the primary and secondary immune responses may be inhibited, adequate levels of immunoglobulins presumably having the usual spectrum of antiviral and antibacterial activities are maintained. These patients are thus somewhat comparable to agammaglobulinemic persons who are treated with replacement γ globulin. In both, immune responses cannot be elaborated, but bacterial infection does not occur because "normal" immunoglobulin levels are adequate.

By contrast, recipients of renal allografts are often stricken by serious infections.[39,40] The reasons for this difference from the patient with immunologic disease treated with cytotoxic drugs are not entirely clear. Multiple immunosuppressive agents are frequently used simultaneously or sequentially with transplantation. These may have cumulative effects and combine to obliterate all or most of the defense mechanisms. As indicated above, many of our patients had at least *part* of their immunologic system intact (IgM synthesis, delayed hypersensitivity or normal total immunoglobulin levels). Furthermore, the majority of them had normal leukocyte counts. It is conceivable that the many months of renal failure and chronic dialysis preceding the actual transplantation procedure can deplete a patient of important physiologic resources – an additional consideration not present in the patient with autoimmune disease.

It thus appears that, when used cautiously, azathioprine and amethopterin do not result in a harmful degree of immunosuppression in patients with immunologic diseases. Nevertheless, the long-term effects of these drugs on the immune apparatus still require evaluation.

Is the induction of bone-marrow depression necessary for immunosuppression? Although leukopenia may occur, particularly with azathioprine, this is not required for immunosuppression. Whether the maintenance of mild leukopenia is necessary for clinical effectiveness of these drugs is debatable. Our experience with amethopterin indicates that it is not. However, LaGrue[41] found the induction of mild leukopenia desirable in the treatment of certain types of nephritis and nephrosis.

What is the correlation, if any, between immunosuppression and clinical results? This question is based on 2 assumptions, both of which may be incorrect. The first is that the disease under treatment is caused by antibodies. The second is that the drugs employed act on the disease primarily by reducing antibody synthesis. Our inability to find a correlation between the extent of immunosuppression and clinical effectiveness of azathioprine and amethopterin suggests the following: one or both of these assumptions are incorrect; the disease in question is too far advanced to permit a response, even with full immunosuppression; the technics that we chose to investigate immunosuppression are not relevant to the problem of autoimmunity; or the pa-

tient is actually protected by his immune response, and its inhibition constitutes incorrect therapy.*

The last 2 possibilities can be eliminated. There is no evidence that the synthesis of autoantibodies differs from that of antibodies to KLH, diphtheria toxoid or any other antigen. Furthermore, autoantibodies, when present, disappeared from the blood of the patients undergoing a remission. We obtained no evidence that azathioprine or amethopterin treatment worsened the disease.

We believe, however, that the possibilities regarding correctness of the assumptions and the effect of far advanced disease must be seriously considered. An excellent case in point is our failure to improve any of 7 patients with severe, corticosteroid-resistant renal disease, despite profound immunosuppression in all. At least 2 factors may account for such failures: the disease is irreversible; or it is caused neither by antibodies nor by any other process susceptible to the effects of antimetabolites. The assumption that antimetabolites affect immunologic diseases primarily by reducing antibody synthesis may be incorrect. The purine antagonists also have profound anti-inflammatory effects. First identified in laboratory animals,[31,33] these properties became evident clinically when very rapid effects on "immunologic" diseases were noted. This type of improvement, occurring in some cases within twenty-four hours, was obviously too rapid to be on the basis of suppression of antibody formation. The observation that 2 patients responded rapidly and dramatically to amethopterin while synthesizing abnormally *high* titers of antibodies seems to support this contention. Whether or not antimetabolites interfere with the pharmacologic mediators of immune injury (for example, serotonin, histamine and bradykinin) or the complement system is unknown. Such effects may have clinical implications, and an investigation of this possibility seems desirable.

The clinical effectiveness of antimetabolites in immunologic diseases thus appears exceedingly complex and probably depends on many factors, one of which is suppression of one or more modalities of the immune response (such as circulating antibodies or delayed hypersensitivity). If a given lesion is produced by a delayed hypersensitivity mechanism (for example, contact dermatitis), suppression of humoral antibodies would be irrelevant. Another factor is anti-inflammatory action. If the expression of immunity (for example, an Arthus reaction) can be selectively blocked by a peripheral, anti-inflammatory effect, central inhibition of antibody synthesis would be unnecessary. The state of the lesion should also be considered: if morphologic and functional integrity is beyond repair, no amount of immunosuppression will cure the defect.

*It is also possible that the antimetabolite therapy was unrelated to the clinical remissions. Evidence that clinical responses occurring in patients given antimetabolites result from that treatment will be presented in a subsequent paper.

The nature of the injurious antibody is important. If the lesion-producing immunoglobulin is IgM (for example, a cold agglutinin), resistance to chemotherapy may be encountered, even though other immunologic responses are inhibited. Furthermore, if the disease in question is not caused by antibodies, delayed hypersensitivity, or an "inflammatory" process, suppression of these modalities may be useless.

Finally, in the light of these arguments, the last question – *"Can measurement of a defined immune response be used as a guide to therapy?"* – has a negative answer unless the precise mechanism of the disease under treatment is known. Unfortunately, in most cases it is not.

SUMMARY

Twenty patients undergoing immunosuppressive therapy (azathioprine or amethopterin) for immunologic diseases were studied. Primary and secondary responses, delayed hypersensitivity, immunoglobulin levels and isoagglutinin titer were investigated. The immune responses were abnormal in all patients. Enhanced antibody synthesis occurred in 3 cases. Selective inhibition of γG-antibody formation was found in 13 patients. The anamnestic response and delayed hypersensitivity were depressed during treatment. Keyhole-limpet hemocyanin was found to be a useful antigen in evaluating immunity in man. The results of these immunologic studies were compared with the clinical responses to the drugs.

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BYSSINOSIS IN THE UNITED STATES*

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BYSSINOSIS is an occupational respiratory disease of cotton, flax and hemp workers. Its initial symptoms of tightness in the chest, dyspnea and cough shortly after return to work on Mondays, after an absence from work during the weekend, are characteristic and stereotyped. The symptoms are accompanied by a decrease of ventilatory capacity during the working day. Later, symptoms may extend to other workdays, and finally there is severe and continuous dyspnea, chronic cough and permanent ventilatory insufficiency. Total disability and death may follow. In the cotton industry, byssinosis occurs primarily among cardroom workers. The acute, reversible effects of cotton dust – that is, the asthma-like Monday symptoms – can be reproduced in healthy subjects by inhalation of cotton dust or aqueous cotton-dust extract aerosol[1,2] and also by inhalation of an extract of bracts, a component of the cotton boll that contaminates raw cotton.[3] Among workers at risk one can identify "reactors" and "nonreactors" – that is, those who respond acutely to textile dust exposure (with symptoms and changes in lung function,) and those who do not. About 70 per cent of all healthy subjects,[2] as well as hemp workers,[4] were "reactors" in this sense.

Byssinosis in the cotton industry was considered a negligible problem in many countries until its true prevalence was established. In Great Britain Schilling et al.[5] showed that 40 to 50 per cent of all workers in cotton cardrooms spinning coarse yarns might suffer from the disease. At present, more than 200 new pensions for total or partial disability caused by byssinosis are awarded annually in Great Britain. In the Netherlands and in Sweden working conditions in mills were thought to be better than those in Great Britain. General pollution of outside

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