enic substrate analogs is observed not only *in vitro* but also in cultures of *E. coli*.[43] What appears to be an interference with a single step in a metabolic pathway leads to an early arrest of bacterial growth. Oleic and vaccenic acids, products whose synthesis is blocked by the acetylene, overcome the action of the inhibitor and restore bacterial growth to normal. These results prove two points: (1) a long-chain unsaturated fatty acid is essential for the growth of *E. coli* (see also ref 40), and (2) the dehydrase is the only inhibitable enzyme in the bacterium. Other bacterial species which synthesize unsaturated fatty acids by the dehydrase-mediated mechanism are equally sensitive to the acetylenic analogs.[44] However, the effect is one of selective bacteriostasis and not of general toxicity. Yeast and animal cells in tissue culture, which produce long-chain unsaturated fatty acids by oxidative desaturation and hence lack the target for the inhibitor, grow normally in the presence of the acetylenic compounds.[43]

A powerful and selective inhibitor of a bacterial enzyme is potentially a useful antibacterial agent. Unfortunately, however, 3-decynoyl-NAC does not promise to be of practical value for chemotherapy. It fails to protect animals against bacterial infections, presumably because of rapid inactivation by serum.[44] Nevertheless, the case of 3-decynoyl-NAC is an example of a more systematic approach to the development of antibacterial agents which utilizes the greatly expanded knowledge of intermediary metabolism and of comparative enzymology to pinpoint appropriate targets for antimetabolites.

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Work described here was supported by grants-in-aid from the U. S. Public Health Service, the National Science Foundation, the Life Insurance Medical Research Fund, and the Eugene P. Higgins Trust Fund of Harvard University. I acknowledge with appreciation the contributions of the graduate students and postdoctoral fellows whose work is summarized in this review. I also wish to thank Mrs. B. Talamo and Mr. George Helmkamp for a critical reading of the manuscript.

The Role of Antimetabolites in Immunosuppression and Transplantation

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Immunological reactions are an essential part of an organism's defense mechanisms against foreign invaders. These reactions work to the advantage of the individual in some situations, *e.g.*, the synthesis of antibodies to combat infectious disease, but may have undesirable and even fatal consequences in others. Examples of the latter are the anaphylactic shock produced in sensitized individuals on further exposure to the antigen which produced the sensitization, *e.g.*, horse serum proteins, and the "autoimmune" diseases (such as autoimmune hemolytic anemia and lupus erythematosus) in which the organism seemingly becomes confused in his discrimination between self and nonself. Tissue and organ transplantation have been technically feasible for many years, as shown by the indefinite survival of autografts (reimplanted in the same individual). However, allografts (from another individual of the same species) are rejected rapidly and become nonviable within a matter of days. It is evident that the ability to control the immune response would enable the physician to deal effectively with a number of serious medical problems including, but by no means limited to, the rejection of a transplanted organ.

Considerable progress has been made in the discovery of drugs to control the immune response. Some of the most effective drugs are antimetabolites which interfere with the normal metabolic pathways involving nucleic acids. This Account will concentrate on the clues that studies with antimetabolites, and in particular with thiopurine derivatives, have provided with respect to immune mechanisms. These compounds have provided the point of departure for transplantation and for much investigative work on basic immunology. A number of reviews that cover immunosuppressive agents more broadly are available.[1-3]

Features of the Immune Response

The small lymphocytes make up about 20% of the white cells in the circulating blood of man. They are the major cellular type in lymph nodes, thymus, and spleen (lymphoid tissues) and are also distributed diffusely in other tissues, such as bone marrow and intestinal mucosa. Unlike other leukocytes, they are neither phagocytic nor chemotactic, nor do they divide continuously.

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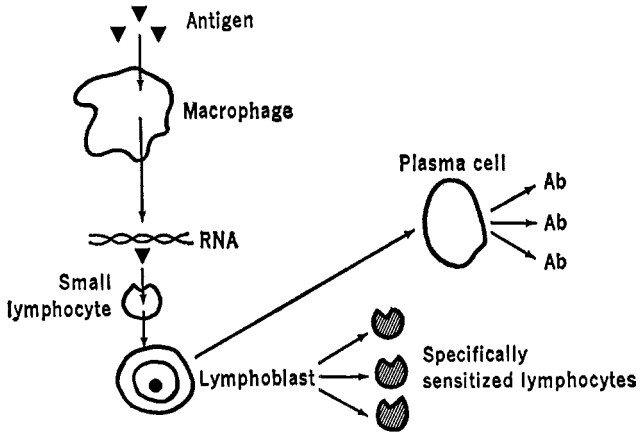
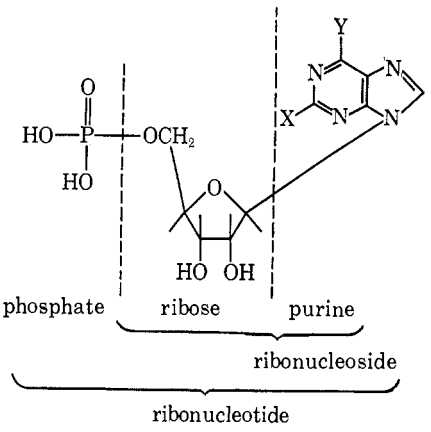


Figure 1. Hypothesis for the mechanism of sensitization of small lymphocytes by antigen. The antigen is processed by the macrophage where it acquires an RNA component and is then transferred to a small lymphocyte. The lymphocyte is converted to a primitive form, the lymphoblast. Some lymphoblasts differentiate into plasma cells (antibody forming), while others give rise to sensitized small lymphocytes.



	Purine	Ribonucleoside	Ribonucleotide
X = H, Y = OH	Hypoxanthine	Inosine	Inosinic acid
X = H, Y = SH	Mercapto-purine	Thioino-sine	Thioinosinic acid
X = H, Y = NH_2	Adenine	Adenosine	Adenylic acid
X = NH_2, Y = OH	Guanine	Guanosine	Guanylic acid

Figure 2. Illustration of the relationship of a purine base to its ribonucleoside and ribonucleotide, and the substituent groups and names of some of the compounds discussed in the text.

The mechanism of initiation of an immune response is not understood in ultimate detail. A foreign substance capable of evoking an immune response (*antigen*) is recognized as nonself by a small proportion of the lymphoid cells exposed to it. Responsive cells undergo a series of transformations that ultimately result in cells committed to the synthesis of a specific antibody or in specifically sensitized small lymphocytes or both (Figure 1). Both transplant rejection and delayed hypersensitivity reactions are characterized by invasive accumulation of small lymphocytes. In transplantation their target is the transplant itself; in delayed hypersensitivity it is the region surrounding the spot of injection of an antigen.

It is currently believed that two distinct cell lines may participate in immune reactions. Gut-associated lymphoid tissue is believed to give rise to cells primarily concerned with the production of circulating antibodies, while cells differentiated in the thymus may be responsible for delayed hypersensitivity and the rejection of grafted tissues. This distinction may not be as clear-cut as was once supposed, in view of recent demonstration that circulating antibodies may precipitate a rejection of autografts that involves all the classical features of invasion by sensitized lymphocytes.[4,5]

Identification of lymphoid tissues as the seat of immune reactions brought the target for immunosuppression into focus. The sensitivity of these tissues to such agencies as X-irradiation, alkylation agents, and steroids permitted a broad, unspecific suppression of immune reactions which was helpful in autoimmune disease, but proved ineffective in the more difficult challenge of organ transplantation.

A major advance toward immunosuppression was taken by Schwartz and Dameshek,[6] who provided the first evidence of drug-induced immune tolerance, using 6-mercaptopurine (6-MP), an antimetabolite which previously had become established as an antileukemic agent.[7] It has now been supplanted by its near relative, azathioprine (Imuran), in transplantation and autoimmune disease, but both have been used to advance knowledge of the phases and cellular events in the immune response.

Biochemical Loci of Action of 6-Mercaptopurine and Azathioprine

Mercaptopurine arose from a program designed to create analogs of the nucleic acid bases and to study their ability to interfere with nucleic acid biosynthesis.[8,9] At the time that mercaptopurine was synthesized and its antileukemic activity discovered, the pathways for the biosynthesis and incorporation of purines into nucleic acid were largely unknown. However, it was apparent that 6-MP was a specific antagonist of hypoxanthine. The elucidation of the biochemical pathways of nucleic acid synthesis revealed that the ribonucleotide of hypoxanthine, inosinic acid (Figure 2), is not only the first biosynthetic purine, but the core of all purine metabolism. Mercaptopurine imitates hypoxanthine in many respects and consequently becomes involved in many steps of purine anabolism. The critical locus of action, if

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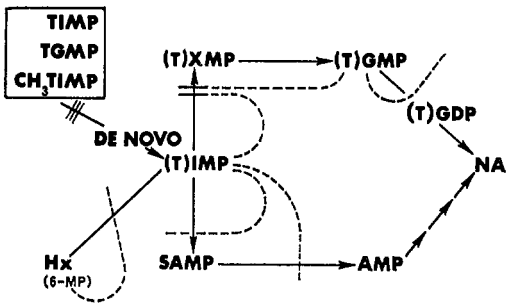

Figure 3. Scheme for purine interconversions and the loci of action of 6-mercaptopurine (6-MP) and its derivatives: (T) = thio; Hx = hypoxanthine; IMP = inosinic acid; XMP = xanthylic acid; GMP = guanylic acid; GDP = guanosine diphosphate; SAMP = adenylosuccinic acid; AMP = adenylic acid; NA = nucleic acid; CH_3TIMP = 6-methylthioinosinic acid.

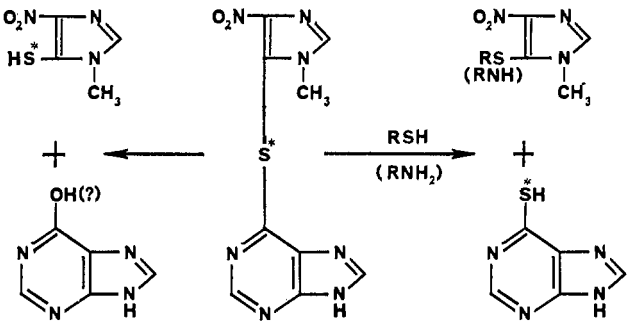

Figure 4. The cleavage of azathioprine-^{35}S (6-(1-methyl-4-nitro-5-imidazolyl)thiopurine) by sulfhydryl compounds (RSH) or amines (RNH_2) to give 6-mercaptopurine and by an unknown mechanism to give 1-methyl-4-nitrothioimidazole-5-^{35}S (*S = ^{35}S).

indeed there is only one critical locus, still remains a matter of debate.

Some of the pathways which mercaptopurine and its derivatives are known to inhibit are shown in Figure 3.[10-12] Mercaptopurine is a substrate and a competitive inhibitor of hypoxanthine phosphoribosyl transferase and is converted by this enzyme to thioinosinic acid. Thioinosinate, in turn, inhibits the conversion of inosinate to both adenylate and guanylate. Moreover, thioinosinate is apparently converted to thioguanylate, since administered mercaptopurine has been identified in DNA in the form of deoxythioguanosine. Thioguanylate is a known inhibitor of several of the anabolizing enzymes, *e.g.*, inosinate dehydrogenase and ATP:GMP phosphotransferase.[13] In addition, the first step of purine biosynthesis, the formation of phosphoribosylamine from glutamine and phosphoribosyl pyrophosphate, is subject to feedback inhibition not only by the natural nucleotides, adenylate and guanylate, but also by thioinosinate, thioguanylate, and particularly 6-methylthiopurine ribonucleotide (a further metabolite of thioinosinate *in vivo*).[14,15] From the reactions discussed it is apparent that mercaptopurine can inhibit RNA and DNA synthesis, but these studies do not come to grips with the main point, the reason for the drug's selective effects.

Azathioprine (Imuran) (Figure 4) evolved from studies of substituted mercaptopurines and was conceived as a "masked" form of mercaptopurine which might permit the delivery of mercaptopurine to target sites where it might then be released.[16,17] The compound was found to be cleaved readily by various sulfhydryl compounds *in vitro*,[16] with the release of mercaptopurine. When these studies were carried out with azathioprine-^{35}S and hydrogen sulfide, the cleavage was found to occur so that the ^{35}S was found exclusively with the purine moiety (Figure 4). *In vivo*, azathioprine-^{35}S is likewise split to mercaptopurine-^{35}S but, unexpectedly, a small amount of the labeled sulfur was also found as 1-methyl-4-nitro-5-thioimidazole[18] (Figure 4). The mechanism of the latter reaction remains to be elucidated. Current investigations suggest that amines, as well as sulfhydryl compounds, can participate in a nucleophilic attack on the nitroimidazole portion of azathioprine.[19] Although the substituted imidazoles thus formed do not in themselves seem to be immunosuppressive, it is conceivable that depletion of important sulfhydryl or amine groups may occur in the process of cleavage. This could give azathioprine a locus of action in addition to that of the mercaptopurine which it releases. Comparisons of azathioprine and mercaptopurine in several immunological systems suggest that, in some of these, azathioprine has a better chemotherapeutic index (maximum tolerated dose/minimum effective dose); it is generally regarded as preferable for organ transplantation and autoimmune disease.

Characteristics of Immunosuppression

A. Circulating Antibody. The agencies (radiation, alkylating agents, steroids, etc.) which owe their ability to suppress the immune response to a reduction in the total mass of lymphoid tissue are maximally effective when given several days before the antigenic stimulus, so that a depletion of the responsive tissue has already occurred when the challenge is presented. The temporal relationships are quite different when antimetabolites are used for suppression. This was first

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shown by Schwartz, Stack, and Dameshek[20] using bovine serum albumin in rabbits and various dosage regimens of 6-mercaptopurine (6-MP). Treatment with the drug prior to the injection of bovine serum albumin had no effect on subsequent antibody formation. Treatment after the antibody response had become well established was likewise ineffective. However, when 6-MP was administered during the induction period of the immune response, *i.e.*, starting at the time of antigenic stimulus and continuing for a short period thereafter, a prolonged tolerance could be attained. Moreover, tolerance induced in this way was specific for the antigen used.[6] Thus, a rabbit could be made tolerant to human serum albumin by treatment with 6-MP for 2 weeks following human serum albumin administration. After 30 days, the rabbit, rechallenged with human serum albumin in the absence of drug, failed to make antibody to this antigen. However, a perfectly normal antibody response to another antigen, bovine γ-globulin, could be elicited. A number of other workers[21-23] have brought the characteristics of timing and specificity into even sharper focus by the use of single doses of immunosuppressive agents.

Drugs also are capable of producing discrimination among types and phases of the immune response. In the primary response to an antigen, the antibody which appears first is a macroglobulin, IgM, with a sedimentation constant of 19S. This is ordinarily replaced after some days by a lower molecular weight γ-globulin (7S IgG). When the animal is rechallenged with the same antigen, it produces antibody more rapidly than after the initial stimulus, and the antibody, almost from the beginning, is of the 7S IgG type. With subsuppressive doses of 6-MP it has been possible to block 7S antibody formation, while the synthesis of 19S antibody persists[24,25] (Figure 5). (Injection of 7S antibody into such animals inhibits the production of the 19S globulin, suggesting some kind of feedback control of 7S upon 19S production).[24,26,27] Since it is 7S antibody which appears to be associated with immunological memory,[24,26] the blocking of 7S antibody production leads to a state in which subsequent rechallenge with antigen gives rise to a primary rather than a secondary response. This sort of "partial tolerance" can be found in several early reports on the action of immunosuppressive drugs.[1,28]

The suppression of a secondary response by mercaptopurine is much more difficult than that of a primary response.[29] Nevertheless, by the use of large doses of both drug and antigen at the time of the secondary challenge it is possible to obliterate the "memory" which the primary response to antigen has established.[30]

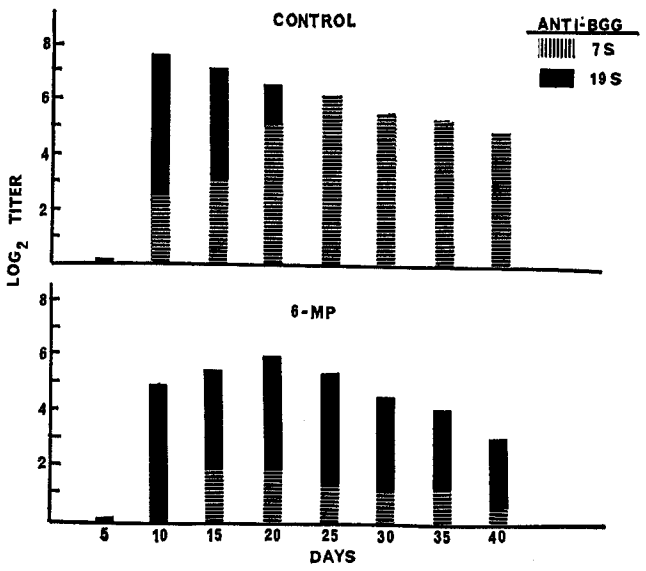

Figure 5. The circulating antibody response of rabbits at various times following a single injection of an alum precipitate of bovine γ-globulin into the footpad. Animals treated with 6-mercaptopurine received 10 mg/(kg/day) subcutaneously for 7 days following the antigen. The two types of antibody, 7S and 19S, were determined by sensitivity to 2-mercaptoethanol, sucrose gradient untracentrifugation, and chromatography on Sephadex G-200 (data from ref 24).

A factor of major importance in the production of drug-induced tolerance is the dosage of antigen. Suppression is increased, rather than diminished, by increasing the antigenic stimulus. This was found in our laboratory during early explorations of dose–response relationships employing a sheep erythrocyte–mouse system.[31] Suppression with azathioprine was greater not only when larger doses of drug were used, but also with the larger doses of antigen (Figure 6). This relationship of immunosuppression to the dosage of antigen was also shown by Schwartz[32] for tolerance in rabbits to bovine serum albumin and by Brooke[33] for the production of immune paralysis in mice to pneumococcal polysaccharide. This finding may indeed be a clue of major importance in understanding how immunosuppressive drugs act, and how specificity for a given antigenic stimulus can be attained through drug action.

B. Delayed Hypersensitivity and Autoimmune Disease. Delayed hypersensitivity, a manifestation of cell-mediated immunity, can be suppressed by drugs.

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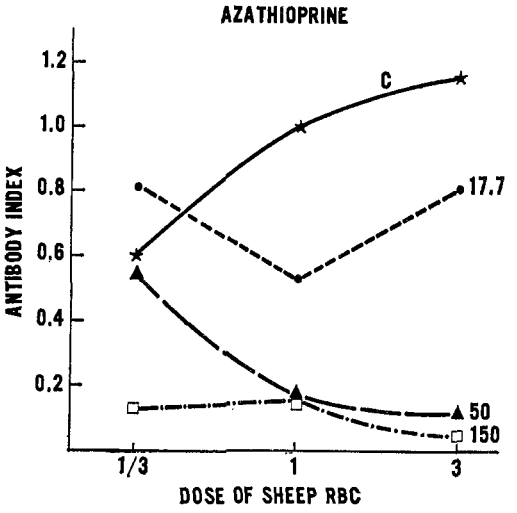

Figure 6. The hemagglutinating antibody response in mice 14 days after a single injection of varying amounts of sheep red blood cells. The unit dose of sheep RBC was 0.5 ml of a 30% suspension in saline. Azathioprine was given intraperitoneally at the indicated doses (mg/(kg/day)) for 4 days following the sheep red cell injection. The antibody index is based on a comparison of the antibody titer with the titer obtained when the control animals were given 1 unit dose of sheep RBC.[31]

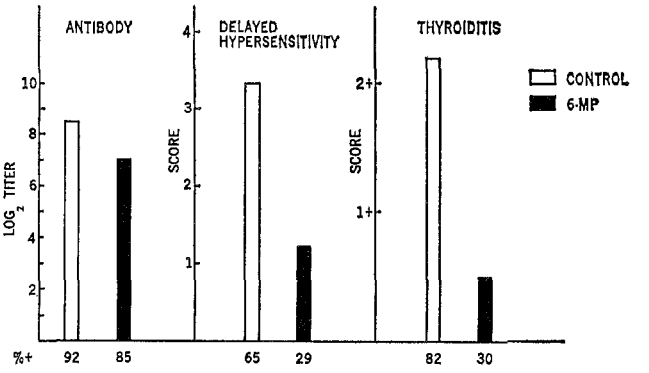

Figure 7. Mean values for serum antibody titer, delayed hypersensitivity (skin infiltration), and thyroiditis in guinea pigs immunized with thyroid extract. Antigen (thyroid extract protein + complete Freund's adjuvant) was injected in the footpads on days 0 and 4. The experimental group was treated with 6-mercaptopurine, 150 mg/(kg/day) intraperitoneally, from day 0 to day 19. The skin test was done on day 20 and the experiment was terminated on day 21.[36] The percentage of animals showing a positive response is shown below each bar.

This applies not only to delayed skin reactions[34-36] but also to experimentally induced autoimmune diseases, *e.g.*, autoimmune thyroiditis,[36] experimental allergic encephalomyelitis,[37-39] adjuvant arthritis,[38-40] and nephrotoxic nephritis.[41] Indeed, drugs may repress cell-borne immunity without a comparable inhibition of circulating antibody responses. Thus, Spiegelberg and Miescher[36] suppressed autoimmune thyroiditis and delayed hypersensitivity in guinea pigs with doses of mercaptopurine which scarcely affected circulating antibody (Figure 7). This may represent prevention of the sensitization of lymphocytes, but some reservations have to be maintained in view of the demonstration that azathioprine, at least, may have a direct effect on cell–cell interactions, *e.g.*, between sensitized rat lymph node cells and target lymphoma cells *in vitro*.[42]

C. Organ Transplantation. Organ transplantation has been more difficult to understand and categorize than the simpler immunological models. Canine renal allografting, using 6-mercaptopurine as an immunosuppressant, was begun by Calne[43] shortly after Schwartz and Dameshek's observations were first published. These experiments produced significantly prolonged survival of grafts; azathioprine seemed an improvement[44,45] and was chosen for initial experiments in human renal transplantation.[46]

Some specificity has been demonstrated with respect to graft tolerance. Thus, a dog tolerating a kidney graft was capable of rejecting skin from the kidney donor.[28,45] Differential rejection of one of two grafts from different donors was possible, demonstrating that the challenge (or histocompatibility) might vary in different pairings.[45] A few dogs (and at least one human)[47] have become tolerant of their grafts so that treatment could be withdrawn after prolonged periods,[28,48,49] but the animals were shown to be simultaneously sensitized, since a second graft from the same donor was rejected in accelerated fashion,[28,50] and in one case the first (apparently tolerated) graft also succumbed to the reactivated immunological assault.[50] So far, attempts to facilitate tolerance to kidney grafts by increasing the antigenic stimulus have been unproductive, but perhaps the only permissible conclusion is that it has not yet been possible to identify the important antigens. In any case, it is clear that graft tolerance is by no means as readily attained as tolerance to simpler antigens, and, in general, the suppression of graft rejection is only achieved through continued treatment with immunosuppressive agents.

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Theories of the Nature of the Immune Response

There have been two main streams of thought about the nature of the immune response. These are grouped as "clonal selection" on the one hand, and "template' ("informational") theory on the other. The one holds that every given lymphoid cell is predetermined in some way to respond to a single antigen with the production of specific antibody. Template theory holds that the antigen somehow directs the synthesis of the specific antibody that combines with it. There are elements of both predestination and free will in the immune response. Recognition seems to be predetermined since only a minute fraction of a lymphoid cell population seems capable of responding to any given antigen, and immune tolerance to one antigen may leave normal responses to all others. Furthermore, it seems highly probable that cells, or cell clones, produce only single (or at most two) antibodies. On the other hand experience does alter the immune response. Even during the primary response the nature and affinity of antibody for antigen becomes more specific, and the secondary response is qualitatively and quantitatively more vigorous than the primary.

Current views of protein biosynthesis (Figure 8) hold that a segment of the cells' DNA is transcribed as a messenger RNA which itself is translated into protein on the ribosome, and that the protein formed faithfully represents the genetic code. There is no place in such a scheme for a directive effect of an exogenous protein or other antigen. But those who are true believers in both the clonal selection and the one gene–one protein faiths are faced with enormous difficulties in reconciling the two. To produce the thousands of clones necessary to provide for all the antibodies an organism may some day be called upon to produce, one has to postulate a looseness of character in the differentiating lymphocyte which is not permissible in other cells of the body. There seems to be no reason to suppose that the undifferentiated lymphocytes' genetic material is not orthodox, and the fully differentiated antibody-producing plasma cell seems to replicate itself accurately, since it faithfully turns out the same protein generation after generation, as witness plasma cell tumors. The diversity of potential immunocytes could then be produced only by some drastic shake-up of genetic material that occurred somewhere in the process of differentiation, and then subsided. There is neither direct evidence nor a coherent rationale for such events. It is not unduly difficult to postulate mechanisms by which diversification could have occurred, through mutation, replication, recombination, and crossovers of genetic materials.[51–53] In an evolutionary sense these hypotheses account plausibly for the subunit composition of antibodies[54] and the increasing specificity and diversity of immune responses in higher species. They do not seem to come to grips with the core problem, however, of the apparent multiplicity of cell clones within a given individual.

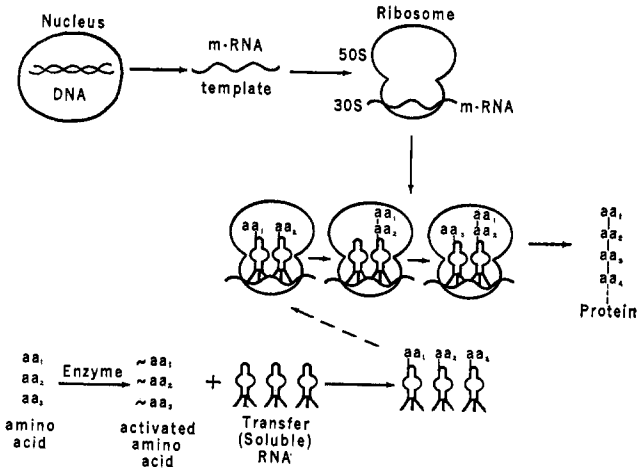

Figure 8. Present theory of protein synthesis. Messenger (template) RNA (mRNA) is synthesized on a DNA template. Ribosomes (polysomes) are attached to mRNA through their 30S subunit. Each activated amino acid reacts with its specific transfer RNA (tRNA) to form aminoacyl-tRNA. A single codon, a triplet, in the mRNA directs specific binding of the aminoacyl-tRNA (containing the anticodon) to the ribosome. Polymerization of the aminoacyl moieties into a peptide occurs on the surface of the ribosome (polysome).

Instructional theory is even more remote from a rational, factual, mechanistic explanation of the immune response. Some latitude in codon recognition (translation) has been demonstrated for certain systems; this is thought of as "wobble" in the fit of transfer RNA to the messenger–ribosome complex so that the third base of the codon of the messenger might be read by several different bases in the anticodon, with quite different results.[55] Translation can also be modified systematically and reproducibly by substances (*e.g.*, streptomycin) that combine with the ribosome.[56] Other means by which an exogenous substance could modify the transcription and translation of the genetic message could be postulated, but none of these has the elements of stop–start, and ultimate locking, fixation, and memory that would be required of a coherent theory of immunology.

Effects of Antimetabolites on Various Stages of the Immune Response

The knowledge that an antimetabolite can inhibit various steps in the biosynthesis of RNA and DNA does not answer the salient question as to how such compounds may exert an immunosuppressive effect. One viewpoint is that these compounds are simply inhibitors of DNA synthesis and, therefore, of cell multiplication, and that a rapidly dividing clone of

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cells is preferentially inhibited. But this is only a broad cloak to hide our ignorance. There are more specific loci which can be postulated. For many of these, the evidence is still be gathered. However, the present ability to stimulate the immune response *in vitro*[57] may now make such biochemical studies more feasible.

The first step in antigen recognition may consist in processing of antigen by macrophages. This appears to involve the synthesis of RNA or, at least, the addition of RNA to antigen, since the signal developed in the macrophage and passed on to the small lymphocyte is sensitive to ribonuclease.[58,59] If such RNA synthesis were inhibited, the small lymphocyte might never be sensitized.

Thiopurines are known to inhibit cell differentiation in embryonic tissue;[60] it is not unlikely that they could also inhibit the differentiation of a small lymphocyte to a lymphoblast.

The synthesis of antibody is, after all, only the formation of a specific protein, and the process of protein synthesis could be vulnerable at many stages to nucleic acid antimetabolites. There could be interference with the formation of template (messenger) RNA, or a nonsense template could be formed which would lead to the synthesis of an inactive protein. 8-Azaguanine is incorporated into messenger RNA, but this does not interfere with the ability of the messenger to function.[61] One does not yet know whether mercaptopurine is incorporated into messenger RNA. The inhibition of induced enzyme formation by purine and pyrimidine antagonists is known. In the induced synthesis of β-galactosidase, for example, 5-fluorouracil has been shown to be responsible for the formation of a nonfunctional, "nonsense" protein.[62] That azathioprine can have a preferential effect on the synthesis of antibody as compared with the synthesis of total protein is shown by the results of Leung and Vas[63] with hyperimmunized rabbit lymph node fragments *in vitro*.

Transfer (soluble) RNA, which is involved in protein synthesis, is another possible target for inhibition, although the evidence to date suggests that this is not a sensitive locus. Soluble RNA containing 8-azaguanine has been shown to function normally in amino acid transfer.[64,65] In our laboratories[66] the soluble RNA isolated from the livers of rats dosed chronically with mercaptopurine showed no change in amount, degree of methylation, or ability to accept the amino acids valine and tyrosine. Since guanosine triphosphate (GTP) is required as a coenzyme for the acceptance of activated amino acids by soluble RNA, it is tempting to speculate that thioguanosine triphosphate might inhibit this reaction. However, such inhibition did not occur with either thioinosine triphosphate or thioguanosine triphosphate, and azaguanosine triphosphate could even replace GTP as the coenzyme.[67]

Still unexplored are the effects of the thiopurines on ribosomal RNA. This site seems less likely, however, since the synthesis of many other proteins proceeds normally in animals in which antibody synthesis is blocked by immunosuppressive agents.

From a practical standpoint, antibody synthesis may be too late in the chain of events of the immune response to achieve the specificity and selectivity that are required of useful immunosuppression. Protein biosynthesis is, after all, a function of nearly all living cells. More promising targets would appear to be the cells involved in recognition and the very early stages of initiation, for such cells would appear to be involved in activities that are uniquely a property of immunologically responding cells. Knowledge of the events of the induction period is still limited, and few clues are available as to what the biochemical events might be. Induction seems to require a fixed time that is relatively independent of the number of cells. It seems clear that DNA synthesis is a prominent event during induction, but it is not so clear that rapid cell division ensues during the primary response. On the other hand, it is quite clear that rapid cell division is an integral part of the secondary response.[68] This difference in rate of cell division has a bearing on the interpretation of the mechanism of drug-induced tolerance and suppression. It might be argued that, since most of the immunosuppressive drugs are cytotoxic agents known for their action on rapidly multiplying lymphoid cells, their action is simply to destroy any clone or clones that respond to the stimulus. But this falls down since the secondary response (in which multiplication is more rapid) is harder to control than the primary. It fails further in that tolerance to nonliving antigens is impermanent and, when it runs out, sensitized cells remain.[28] This brings forward an alternative to cell destruction as a means of producing unresponsiveness. Antigens produce tolerance to themselves when given to immunologically incompetent animals (*e.g.*, neonatal mice) or when given in amounts that overwhelm the animals' capacity to respond (immune paralysis). In all respects the action of the immunosuppressives seems to be to increase the sensitivity of the animal to this effect of antigen. The specificity of the immunosuppression and the greater

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effectiveness of larger doses of antigen are both explained on this basis. There remain to be developed clearer interpretations of the roles of both drug and antigen in this effect.

Practical Applications of Immunosuppression

Although much remains to be learned about basic immunology from both cellular and biochemical standpoints, the practical applications of immunosuppression have burgeoned. In the brief span of 7 years, kidney transplantation has progressed from a tentative experiment to almost a routine procedure, and several thousand patients with autoimmune disease are benefiting from immunosuppressive therapy. Autoimmune disease is a rather loosely defined category of maladies in which there is some evidence for the production of antibodies reacting against the patients' own tissues or organs. In many instances the origin of such antibodies remains obscure, but in most of these diseases there seems to be an element of self-perpetuation; *i.e.*, as a consequence of the presence of antibody more antigen is liberated, more antibody is then formed, and so on. Benefit from immunosuppressive drugs does not unequivocally establish a "therapeutic diagnosis" since these drugs appear to have "anti-inflammatory" effects that are distinguishable from their effects on immunity.[34,69,70] Whatever the mechanism, they are providing effective therapy for such conditions as lupus erythematosus,[71,72] nephrotic syndrome,[73,74] chronic active hepatitis,[75,76] autoimmune hemolytic anemia,[77] ulcerative colitis,[78,79] Wegener's granulomatosis,[80] and other conditions generally classed as autoimmune in origin.[81]

Well over 2000 human renal transplantations have now been performed,[82,83] and the success rate has steadily improved, so that now 95% of the patients are alive with good kidney function at the end of a year.[84] These improvements have come about through advances in tissue matching,[83] which has reduced the challenge to be overcome, and through the addition to the therapeutic regimen of agents like steroids, and most recently antilymphocytic serum.[83,84] The latter is prepared by the immunization of a suitable animal to lymphocytes, spleen, or thymus of the species of the intended recipient. Antisera, prepared in this way, effectively interfere with the responses of the lymphocytes of the recipient, whether by a kind of passive interference (coating or blindfolding), or by acting as a competitive antigen in which a widespread purposeless activation occurs, or by some still unrecognized mechanism.[85] Antilymphocyte serum may or may not be unique in its mode of action; phytohemagglutinin, for example, produces a widespread nonsense activation of lymphocytes and is also immunosuppressive,[86–89] and many of the selective effects of antilymphocytic serum are similar to those previously demonstrated for immunosuppressive drugs. However, it appears to have a high degree of effectiveness in the suppression of graft rejections and under certain conditions may produce prolonged immunological tolerance.

Finally, it seems certain that both conceptual and practical advances hinge on the solution of a few rather key problems that are progressively coming more sharply into focus.

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