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SPECIAL ARTICLE

Autoimmunogens, Autoantigens, Autoantibodies and Autoimmune Diseases: One-to-one or Many-to-many Relationships?

Peter B. Billings

Autoimmune diseases comprise a bizzare collection of disorders with little apparent in common other than that each is associated in some manner with an immune response in which self components are recognized in an aberrant and exaggerated manner. As it has become more apparent that potentially autoreactive clones and self-recognition constitute normal components of a functional immune system, the central question of "what causes autoimmunity?" has often been broached in the context of "what prevents autoimmunity?" The distinction emphasizes the real problem--that we simply do not know enough at present about the normal functioning of the immune system to understand the full complexity or etiology of even a single autoimmune disease. Nor can we identify common and dissimilar features and weight each in terms of significance and contribution to the disease state. Given the large number of autoimmune diseases and the apparent lack of an underlying similarity, one must conclude that either quite different factors are involved or that common factors have unequal significance in the various autoimmune diseases.

Autoimmune responses, like more customary responses, involve to varying extents, both humoral and cellular aspects. Largely in the interest of brevity, the following discussion will be confined to a few representative autoantibodies (AAb) and their corresponding autoantigens (AAg), despite the fact that in many cases, autoreactive cells are more central to the pathogenetic process. In this review, the question of "What triggers autoimmunity/autoimmune disease;" is approached from a slightly different point of view, namely, "What can be inferred as to the origins of individual AAb (or autoreactive cells) based upon their specificities?" This certainly has a link to the disease since the autoantibodies arise in the context of one or more autoimmune diseases, but exactly what this link is, when and how it was established, or how general it is between diseases or the same disease in different individuals, are difficult to ascertain. To the extent that it is possible then, I wish to play down this disease association, and concentrate on the more accessible relationship between AAb and AAg, the restrictions this places upon the possible immunogens and only hint at possible relationships of the latter to particular diseases.

The most common approach for subdividing and ordering this heterogeneous group of diseases, is to rank them according to the extent to which characteristic autoantibodies are organ/tissue-specific. The diseases then fall on a continuous spectrum ranging from those with readily-identified, essentially organ-restricted AAb, to the rheumatic and connective-tissue diseases, with complex populations of apparently dissimilar autoantibodies which recognize widely dispersed, but often poorly-characterized targets. Diseases at one or the other end of this spectrum have considerable in common, frequently including the overlap of symptoms and some autoantibody specificities. Thus, classic organ-specific diseases such as Hashimoto's thyroiditis, Addison's disease, pernicious anaemia, *etc.* appear to share certain features,

From the Department of Microbiology, Faculty of Science Mahidol University, Bangkok 10400, Thailand.

Abbreviations: AAb, autoantibody; AAg, autoantigen; AcChoR, acetylcholine receptor; CRI, crossreacting idiotype; EBV, Epstein – Barr virus; Id, idiotype; MCTD, mixed connective – tissue disease; SLE, systemic lupus erythematosus; Tg, thyroglobulin; Tdx, thyroidectomy.

and AAb characteristic of different organ-specific diseases are often manifest in the same individual. These disorders are quite dissimilar from those at the opposite end--including systemic lupus erythematosis (SLE), mixed connective-tissue disease (MCTD), scleroderma, rheumatoid arthritis, *etc.* (See Table 1 for a listing of several autoimmune diseases and their characteristic AAb).

Table 1 This table represents but a partial listing of human autoimmune diseases concentrating on those used as examples in the test. No attempt has been made to be comprehensive in listing autoantigens, particularly in the rheumatic and connective-tissue diseases where overlap is frequent.

Disease	Autoantigen
Hashimoto's Thyroiditis	Thyroglobulin (destructive invasion of thyroid, swelling, loss of function)
Primary Myxoedema	Thyrotropin (TSH) Receptors (blocking antibodies, thyroid atrophy)
Graves' Thyrotoxicosis	Thyrotropin (TSH) Receptors (growth and metabolism stimulating autoantibodies)
Pernicious Anaemia	Intrinsic Factor (both blocking and binding Ab preventing vit B_{12} absorption) Gastric mucosa parietal cells
Addison's Disease	Cytoplasmic Ag Adrenal Cortical Cells
Insulin-Dependent or Juvenile Onset Diabetes	Cytoplasmic Ag of Islet Cells
Insulin Resistant Diabetes, with acanthosis nigricans	Insulin Receptor (blocking antibodies)
Myasthenia Gravis	Acetylcholine Receptor (blocking antibodies, receptor loss)
Goodpasture's Syndrome	Basement Membrane (glomerular & lung)
Sjögren's Syndrome	Salivary Gland Duct Epithelium; La/Ro; mitochondrial Ag, Rheumatoid Factor
Rheumatoid Arthritis	IgG (Rheumatoid Factor); EBNA – 1
Scleroderma	Scl-70 (70K nonhistone protein); nucleolar RNA; centromere (marker, C.R.E.S.T.)
Dermato/polymyositis	tRNA, tRNA synthetase (Jo-1); PM – 1; Mi-1
Mixed Connective-Tissue Disease	U1-snRNP (marker); ssDNA
Systemic Lupus Erythematosus	dsDNA; ss/dsDNA; ssDNA; histones; Sm; La; Ro; U1-snRNP; non-histone proteins; nuclear matrix; (essentially entire nucleus); cytoskeleton; IgG (Fc); platelets, erythrocytes; cardiolipin

The very term "autoimmune disease" suggests an obligate involvement of at least some of these autoantibodies or autoreactive cells in the course of the disease, *i.e.* their central if not pivotal role in the pathogenesis. In general, the extent to which autoantibodies can be shown to be implicated in the pathogenesis of the corresponding disease seems to parallel the organ/tissue specificity of the major AAb. In those diseases from the middle of the spectrum, it is an organ-, tissue- or cell-specific subpopulation which is implicated in primary pathogenesis. Evidence supporting direct roles of certain subsets of autoantibodies in pathogenesis has come from various sourses in addition to clinical and histopathological findings, including spontaneous-, or antigen-induced animal autoimmune disease models, and passive transfer of disease with lymphocytes, serum or even monoclonal antibodies.

In contrast to the organ-specific AAb, those associated with the rheumatic and connective-tissue diseases have no known primary pathogenetic roles, but rather, seem to be secondary or epiphenomenal, if not essentially inoccuous. The rheumatic diseases are characterized by a multiplicity of partially overlapping sets or patterns of non-organ-specific AAb, frequently targetted against highly conserved, ubiquitous macromolecules. From the point of view of their abundance and distribution, these AAg include some of the most unlikely targets imaginable, *e.g.*, histones--probably *the* most highly conserved class of all proteins, and DNA--the very genetic material itself. A complex and heterodisperse set of autoantigens are associated with each systemic rheumatic disease, but an individual patient afflicted with *e.g.*, SLE, MCTD, Sjögren's *etc.*, possesses only a relatively small subset of the corresponding autoantibodies. Is there really a basic difference in the pathogenetic roles of the autoantibodies in these diseases?, or does this merely reflect our more limited knowledge of systemic rheumatic diseases, the complexities and true identities of autoantigens involved? Certainly part of the difference could simply derive from the obvious, that it is easier to hypothesize roles in pathogenesis if the autoantibodies are of limited diversity, are restricted to a single or few related diseases and target precisely to the same organs associated with the major disease symptoms. There is little compelling evidence that the AAb in rheumatic diseases directed against common nuclear and cytoplasmic antigens even have access to corresponding targets--that they can penetrate cells and disrupt cellular function. One caveat before dismissing most or all AAb associated with these diseases as pathogenetically inconsequential, is that quite possibly the important autoantibodies in terms of pathogenesis have been overlooked in the presence of an overwhelming abundance and complexity of lesser- or unimportant autoantibodies, or that the most apparent or only autoantigen as yet assigned to an AAb, is not the "real" target involved in the pathogenesis.

There may exist many more diseases with associated autoimmune phenomena, but where the AAb and their involvement have not been suspected or recognized as yet. Autoantibodies detectable by common assays occur in higher than normal frequencies among relatives of those afflicted with autoimmune diseases. These "runs" in families tend to follow the same general trend toward organ/tissue-specificities or more typical rheumatic-like specificities. They range from clinically healthy individuals (perhaps at some increased risk) to full rheumatic-like diseases or polyendocrinopathies and organ-specific autoimmune diseases. Susceptibilities to many autoimmune diseases have been shown to be associated with particular HLA antigens or extended haplotypes, *e.g.* HLA-B27, spondylitis; DR4 rheumatoid arthritis; HLA-B8/DR3, many diseases including Graves' thyrotoxicosis. Class I, II, and III (complement components), as well as allotype (Gm) associations with various autoimmune diseases have been reviewed.[1] It is also quite well accepted that AAb in appreciable titers can be detected in a low but still substantial proportion of matched "normal" individuals with no apparent deleterious effect.[2,3] Although autoantibodies expressed in "normals" are innocuous in themselves, their presence in high titers and their their persistence suggest to many an underlying imbalance or disturbance in immunoregulatory mechanisms which has either instigated this autoimmune response or at least failed to correct or check it. If an assay sufficiently sensitive to individual clonal specificities is employed (hybridoma construction, EBV transformation, B cell mitogens/activators, *etc.*), essentially all individuals possess autoreactive B cells.[4-7]

A question is often raised, particularly in reference to organ-specific diseases with proven pathogenetic AAb, whether the AAb or the disease is foremost--are the AAb cause or consequence? Within the full spectrum of autoimmune diseases, clearly most AAb fall into the latter category as byproducts or epiphenomena. However, such a question, ultimately resolves into nothing more than semantics--when do you being to call a sequence of progressively deleterious conditions "*the* disease"?. Even in those cases such as myasthenia gravis where blocking AAb directed against the acetylcholine receptor constitute the major if not sole pathenogenetic agent and passive transfer of disease may be effected by serum or monoclonal AAb,[8,9] it is still difficult to

assert that the AAb arose spontaneously and initiated the disease. The AAb are but one factor in the disease which occurs in only some susceptible individuals. Additional abnormalities, coincidental and essential contributory factors must pre- or coexist to overcome homeostatic processes and become chronic and self-sustaining. Thus one might suggest that AAb which were an epiphenomenon relative to the first pre-clinical condition or unrecognized disease, directly resulted in a second, more serious but recognizable disease.

Models for the etiology of autoantibodies

Any model for the origin of AAb need not explain all diseases, all variants of a single disease, or even the full AAb complexity in a single individual. However, it should be compatible with all features of the disease in that one individual and if necessary should accomodate whatever additional explanations/models are required to account for the full complement of AAb specificities expressed. The model should provide not only some rationalle for the origin of the AAb, but also for maintenance of the autoimmune state; in most cases, it is the latter which remains most enigmatic. The various autoimmune conditions induced by drugs, mitogens, parasite infections, *etc.*, may provide insight into particular models, especially since early pre-clinical stages can be observed and manipulated, but they are not self-perpetuating and AAb disappear once the inducing agent is corrected or withdrawn. With rare exceptions, the autoimmune response against a single autoantigen is polyclonal--multiple epitopes on a single protein or complex are recognized. Ultimately, the model should account for which epitopes are recognized, and provide a basis for the failure to respond to expected epitopes if an actual immunogen is involved. Ideally, each plausible model would have general relevance despite differences in autoimmune diseases and individual differences. Because our approach is model-oriented, rather than from the perspective of a single disease or specific autoantibody, there will remain unresolved inconsistencies and some duplication. Where different models could each independently account for the emergence of a particular specificity, it is usually impossible to "weight" the various possibilities. If alternative routes could result in essentially the same "disease", how can one decide which path was taken in a particular individual? Presumably, the AAb reflect the immunopathological history of the individual from the pre-clinical to disease state; the difficulty at present is in reading the record.

Sequestered antigen (immunologically priviliged site)

Postulating a sequestered Ag, is perhaps the simplest view of the origin of autoantibodies and in essence suggests that normal tolerance is not an active state but rather a lack of sensitization resulting from no exposure to antigen and no (or few) autoreactive T- or B-cells. Intuitively, it makes sense and certain known AAg derive from sites which should normally be inaccessible to cells involved in immune recognition. Brain, cardiac and sperm antigens and lens protein are the classically cited examples. Thyroglobulin, once postulated to be confined to the acinar cells is now thought to escape in tolerogenic amounts--most autoantigens are probably the same. A lesion of some sort with release of antigen or infiltration of lymphocytes could result in sensitization and production of AAb, *e.g.* anticardiac Ab provoked by a heart attack or autoimmune responses directed against lens protein following cataract surgery. Clearly, certain autoantigens, such as those cited above could escape tolerance and later be presented to an intact immune system, however, such a mechanism must constitute the exception accounting for but a few of the numerous autoimmune conditions.

Polyclonal activation

There is now abundant evidence that normal non-responsiveness is not due to lack of B cells with autoantigen receptors (clonal deletion, forbidden clones, *etc.*). Autoantibody secretion by peripheral blood and splenic lymphocytes from unimmunized donors appears to be the rule rather than the exception when studied *in vitro* using B cell mitogens and polyclonal activators (LPS, peptidoglycan), Epstein-Barr virus infection or myeloma fusion (hybridoma construction), all of which reflect T-cell bypass mechanisms.[4-7] The autoantibody specificities, although not yet verified at the epitope level, appear similar if not identical to their spontaneous counterparts in the systemic rheumatic diseases. Many parasitic infections (trypanosomes, malaria, *etc.*) are associated with a secondary autoimmune phenomenon, and malarial culture supernatants have been shown to contain a mitogen capable of inducing anti-nuclear AAb, rheumatoid factor (anti-Ig) and anti-intermediate filament antibodies in normal lymphocytes.[10] Thus, such a mechanism could be involved in certain autoimmune phenomena. A reversal of the normal T cell balance, *i.e.* lack of supression or excessive help, could have the same effect, and characteristic disturbances of T-cell/B-cell or T-cell subsets are associated with numerous autoimmune diseases.[11] Such imbalances of immunomodulatory cells in turn could be mediated through even more general metabolic alterations (*e.g.* stimulation or inhibition of cyclic AMP).

It is precisely the great diversity of autoantibodies in the systemic rheumatic diseases, that anything and everything can be, and is an AAg in one or another patient, that supports a model for non-specific polyclonal activation.[12]

The questions are then: are we looking at the potential B cell repertoire or the actual autoantibodies expressed in a disease state?, and does this phenomenon extend to the point of a "disease"? Can inherently non-specific mechanisms explain the apparent exquisite specificities and the characteristic overlap or co-expression patterns of individual diseases? Similarly, why are only some AAb and not all expressed in a particular disease and even a smaller subset in a particular patient? The *in vitro* experiments eliminate all checks and balances operative *in vivo* upon the expression of these specificities; even if initiated, would not such autoantibody secretion soon be terminated or supplanted by still other specificities, particularly where the AAb are apparently not involved in self-perpetuating or pathogenetic aspects of the disease? In principle, one would expect the activation (despite the term polyclonal) to be poly- (mono)-clonal, with a low probability of activating the numerous clones required for preciptiating polyclonal autoantibodies. Timing, however, could be an important factor, should the non-specific activation be coincident with a transient conventional polyclonal response directed against a single antigen. This still leaves the question of why and how this response escapes normal suppression and becomes chronic, exaggerated and uncontrolled, *i.e.*, why it should properly be termed an autoimmune "disease".

Is there even an immunogen with structural and functional relation to the apparent autoantigen? A full answer cannot be given at present, however, a tentative answer derives from experiments involving the obese strain (OS) chickens, an animal model of Hashimoto's thyroiditis, in which there is spontaneous production of autoantibodies directed against thyroglobulin (Tg). Removal of the sourse of antigen by neonatal thyroidectomy (Tdx) essentially prevented subsequent production of anti-Tg AAb demonstrating that Ag is required for initiation. That Ag is also required for maintenance was shown when adult Tdx reversed an already established autoimmunity. Finally, Tg from either OS or normal chickens administered to neonatal Tdx OS chickens again elicited anti-Tg AAb, suggesting that if Tg is the immunogen, it is not modified in this strain.[13]

Cross-reacting/modified-self antigens

If most soluble autoantigens do circulate and contact corresponding B cells with autoantigen receptors, the normal failure to produce autoantibodies would suggest either that there are few or no specific T cells capable of recognizing the carrier determinants or that they are tolerized/suppressed. Breaking this non-responsiveness or tolerance might then be as simple as introducing new carrier determinants either brought in on a similar "foreign" antigen or through modification of- or association with self antigen.

Immunization with autologous o heterologous cross-reacting antigen: A model for tissue-specific autoimmunity

There are any number of examples of model autoimmune "diseases" which can be instigated by cross-reacting heterologous antigen or by autologous antigen together with a powerful adjuvant. Experimental allergic encephalomyelitis,[14] induced by deliberate immunization with myelin basic protein, has an almost exact parallel in post-rabies vaccination encephalitis where the culprit immunogen represents contaminating host cell protein from virus propagation. Tissue- specific destructive reactions involving AAb and cell-mediated autoimmunity which have counterparts in spontaneous or ideopathic autoimmune diseases, can be induced by immunization with thyroglobulin, acetylcholine receptors, adrenal extracts, testis, *etc.* In such cases the immunogen is known; in the spontaneous disease, it is not. Hence, it is uncertain to what extent the autoantibodies in the experimentally-induced and ideopathic diseases correspond at the level of epitopes recognized. Monoclonal autoantibodies of both spontaneous and experimental derivation are beginning to be produced and characterized and can be exploited to approach just such questions.[15] In myasthenia gravis and Graves' disease, experimental and spontaneous sourses provide monoclonal AAb falling into characteristic subgroups (*i.e.*, blocking or stimulating) and at present, although the evidence is far from complete at the ideotype level, there is no clear reason to suspect they are not drawn from the same repertoire.[16-18]

Viral infection: Crossreacting Ab and Ab to altered-self

Viruses seem unique in that almost all etiological models of autoimmunity could accomodate a role of viral infection in one way or another. This multiplicity of possible roles makes the transition from general models to specific examples difficult. Thus, despite their almost universal acceptance as possible etiological factors, exactly which viruses and what diseases fall into this category, and the precise mechanisms by which viruses might elicit AAb remain largely controvercial. Viral infection would provide a

rich sourse of new and altered carrier determinants, both directly, and through expression of viral antigens on the cell surface or in association with a multitude of host proteins. Perhaps the most direct model, which would also pertain to toxins, bacteria, *etc.*, postulates simply that antiviral antibodies crossreact with host protein(s), located at any site and bearing no relationship with the immunogen--*i.e.*, a true and direct crossreaction. This is analogous to the relationship between the ABO antigens and *E. coli*, although there is no evidence of any autoimmune involvement in the latter. As a viral example, post-infectious encephalomyelitis represents a rare condition in which T lymphocytes are sensitized during rubella, measles, and varicella infections, and react equally with viral antigens or myelin basic protein. Here, computer searches have identified homologous peptide sequences possibly involved in the cross-sensitization, postulated as an early event in multiple sclerosis.[19] In other cases the immunogen may not be a viral antigen proper, but rather a host element rendered immunogenic through association with a viral component or a virus-induced modification--the classic "altered-self". The resulting AAb (cross)react with unmodified self. The major focus of immunological lesions need not coincide with the target tissue, for the same- or still another crossreacting protein could be more accessible, abundant or susceptible elsewhere.

In either of the above cases, should the Ab response prove inadequate or ineffective in viral elimination, a persistent or recurrent infection could sustain a prolonged AAb response--another characteristic of autoimmunity. Virus-induced cell death and release of potential autoantigens could contribute to the establishment and maintenance of the autoimmune state--as could an immunological attack on viral-infected host cells. Other mechanisms involving viruses have been postulated, including polyclonal activation (*e.g.* EBV), virus or viral-response induced alteration in the T cell/B cell balance, induction of interferon, altered or inappropriate HLA-II expression, *etc.* Despite the circumstantial evidence and numerous postulated models, there is little conclusive evidence that specific viruses cause individual autoimmune diseases. One known example of a viral-induced autoimmune disease, however, is the polyendocrinopathy with associated mild diabetes and growth retardation, resulting from reovirus infection of mice. Various monoclonal AAb specific for pancreatic islets cells (some insulin and some glucagon specific), anterior pituitary (some absorbed by growth hormone), gastric mucosa, or unidentified general nuclear components were produced through hybridoma technology from these infected mice.[20] The mechanism by which these AAb arose is unknown, however, unlike the post-infectious encephalitis, it is presumably not through cross-reaction as none of the monoclonal AAb react with reovirus-infected cultured cells.

Host cell-specificity or preference, might be expected to be reflected in the tissue-specificity of the autoantibodies induced, discussion of which is deferred to a later section. On the other hand, lymphoid cells might represent more likely host cell candidates in the non-tissue specific systemic rheumatic diseases. The autoantigens in these diseases include nucleic acids: ssDNA, ds/ssDNA (common or cross-reacting determinants), dsDNA, Z-DNA, ssRNA, dsRNA, nucleolar and transfer RNAs, as well as nucleic acid-protein complexes or protein moieties of such complexes: nucleosomes, ribosomes, small nuclear/cytoplasmic ribonucleoproteins (snRNP, scRNP), centromeres, histones, premessenger RNP, nuclear matrix, *etc.* It has been suggested that if one had to select a candidate immunogen in SLE, a prototypic systemic rheumatic disease, nothing less than the whole nucleus would suffice; recently presence of anti-nuclear antibodies were accepted as one of the criteria for SLE.[21] The almost exclusive association of selected autoantibodies with single diseases provides "marker AAb" useful in diagnosis --*e.g.* anti-Sm (30-40% of SLE patients), anti-U1 RNP (90-100% MCTD), anti-Scl-70 (15-20% scleroderma), anti-kinetochore or anti-centromere (70-90% C.R.E.S.T. variant of scleroderma).[22] Other AAb are linked but not restricted to a particular disease, *e.g.* La (50-60% Sjögren's syndrome; 10-15% SLE) and Ro (60-70% Sjögren's syndrome, 30-40% SLE). That specific autoantibodies are characteristically present in one disease, but low or absent in another, despite considerable clinical overlap, remains an enigma. It is tempting, however, to attribute more than simple coincidence to the fact that most autoantigens are either themselves nucleic acids or normal cellular constituents associated with viral nucleic acids or utilized by the virus at various points during viral uncoating, transcription, translation, replication, packaging and release.

Circumstantial evidence for the involvement of virus alteration in enhancing the immunogenicity of normal cellular constituents comes from many sourses, but our discussion will be limited to the "La" and "Jo-1" autoantigens. AAb directed against the La (or SS-B) protein are present in about half of the Sjögren's patients. In normal cells this M_r 50K protein appears to associate at least transiently with an oligo(U)-rich sequence toward the 3′-OH end of newly-made polymerase III transcripts where it is perhaps involved in maturation.[23] Of relevance here, however, is the fact

that in infected cells, virus-encoded small RNAs are associated with the La antigen and precipitated by anti-La sera: the VA RNAs in adenovirus- and EBER 1 & 2 in EBV-infected cells.[24,25] Autoantibodies against tRNAs and tRNA-associated proteins are common in myositis, while most of the actual muscle dammage is probably due to cytotoxic T cells. The AAg Jo-1 is recognized in 25% of the patients, and is almost certain to be histidyl-tRNA systhetase.[26] A viral etiology has been proposed with coxsackievirus B, a myotropic virus high on the suspected list, but what possible connection could there be between this picornavirus infection and anti-$tRNA^{His}$ synthetase? Mathews and Bernstein have reasoned that since RNA genomes of related viruses can be charged with amino-acids (encephalomyocarditis V with Serine and mengovirus with Histidine), perhaps coxsackie V is similarly charged with histidine.[26] Local cell destruction could lead to the release of the synthetase-viral genome complex in a sufficiently altered and immunogenic form to overcome tolerance. Autoantibodies to the corresponding tRNA could arise as anti-idiotypes to a subset of anti-Jo-1 directed against the tRNA-binding site of the synthetase. Thus, the significance of autoantibodies in terms of deciphering etiologies of the diseases in which they are associated, appears related to the function of these autoantigenic components in the normal cell; it is the perturbation or alteration of this function by agents other than the AAb themselves, which suggest a major link to the disease. In organ-specific diseases it may be the AAb themselves which alter this function, whereas in others the AAb may only evidence some prior structural/functional changes but in an imperfect and distorted reflection. Reconstructing this linkage then, will demand a knowledge of the structure/function of each autoantigen. A clear picture still has not emerged in even a single case. However, knowing that Jo-1 corresponds to $tRNA^{His}$-synthetase, the RNP antigen corresponds to the U1 RNA-containing ribonucleoprotein implicated in viral (and host) pre-messenger RNA splicing,[27-29] and La antigen represents a 3′ terminus maturation factor for RNA polymerase III transcripts, *etc.*, do provide us with working models and a framework for investigation.

At present, with the possible exception of the murine reovirus system, there is no conclusive evidence for an etiological role of viruses in the development and maintenance of autoimmune diseases; even in this case the exact mechanism is unknown. Should one accept the premise that viruses may be involved in some, must one postulate a different virus or viral strain for each disease to account for the variation in symptoms and autoantibody specificities? I think the variation in severity and symptoms between individuals in more conventional viral infections as well as the individual immune response to immunization, would dictate that this premise is unnecessary. Individuals are infected at different times, with variations in state of health and resistance, at different ages, and with different genetic backgrounds, predispositions and immunological histories. The virus may persist, become recurrent or be eliminated and may extend to different cell types. One example, which seems particularly pertinent, is EBV infection, where the virus is simultaneously B cell lymphotropic, a transforming virus and a polyclonal activator. Most individuals are infected at an early age in what must be an unrecognized or mild infection and are sero-converted for EBV nuclear antigen and viral capsid antigen for the remainder of their lives, Many of those infected some - what later in life develop self limiting infectious mononucleosis, while others progress to nasopharyngeal carcinoma or Burkitt's lymphoma.[30,31] The variation presumably is host-, rather than viral strain determined.[32] Perhaps consequences are not always benign in the asymptomatic category.

Aberrant or inappropriate expression of MHC class II antigens

Since current evidence demonstrates that potentially self-reactive B cells exist, the fact that AAb are not normally produced in detectable amounts implies either a lack of specific helper T cells or active suppression. To an unknown extent, this might involve an intrathymic purging of autoreactive T_H cells, however, we need not invoke their actual elimination, merely that they fail to be stimulated. Stimulation requires re-expression of antigenic fragments on the surface of accessory cells in conjunction with matched MHC class II antigens (MHC Class II restriction) in the presence of IL-1. MHC class II (HLA-DR, Ia) expression is normally confined to macrophages, dendritic/Langerhans and B cells, however, under certain stimuli and in several diseases, various cells aberrantly or inappropriately express class II antigens. Many of these diseases are associated with autoimmune phenomena: parasitic infections, biliary epithelia in primary biliary cirrhosis, islet cells in type I diabetes, thyrocytes in Graves', *etc.*[33] Cells inappropiately expressing class II antigens could behave as antigen-presenting (although not antigen-processing) cells and might "present" their own surface antigens, which by virtue of their incorporation in the membrane might bypass the processing requirement. For example, $HLA\text{-}DR^+$ thyroid epithelial follicular cells from Graves' disease patients, unlike normal ($HLA\text{-}DR^-$) thyrocytes, have been demonstrated to "present" a virus-specific peptide to cloned peptide specific, HLA-matched T cells, but could not

"process" antigen and stimulate with intact virus.[34] *In vivo* such cells presumably present their own surface antigens to T_H cells, and in other experiments, cloned T4 lymphocytes from Graves' disease thyroids were shown to respond specifically to autologous DR-expressing thyrocytes but not DR-mismatched thyrocytes.[35] Potent inducers of inappropriate class II antigen expression included lectins and gamma-interferon, where the latter as itself a T cell product, could provide an amplification circuit leading to further activation.[33]

An idiotype/anti-idiotype route to autoantibody production

Antibodies directed against sites at or near the antigen-combining sites of another immunoglobulin, or anti-idiotypes, are now accepted as crucial participants in an interlocking immunological regulatory network.[36-38] In this network, each antibody is intimately linked to many others through mutual idiotype (Id) recognition and can be envisioned alternatively as an autoantibody having reactivity to various extents against idiotypes of a set of other immunoglobulins, and as itself an autoantigen recognized by other antibodies. To say that there is any directionality to this mutual complementarity, is in reality but an operational definition and perhaps "odd/even" or "+/−" would be a more appropriate designation than "Id/anti-Id". Some of these interactions could be considered as mimicking foreign antigens--the "internal image" and behave as ligands. Other anti-Id undoubtedly recognize sites outside what we would designate the antigen-combining site, however, *the* antigen-combining site and *the* specificity are also operational definitions. Id/anti-Id interactions will be presented only from the overly simplistic view of immunoglobulins, whereas in reality Id-specific (not properly anti-Id) T cells would be involved.

Network theorists postulate that it is the equilibrium of all these idiotypic--antiidiotypic interactions between cells and between immunoglobulins and cells that up- or down-regulate the various specificities. Some immunoglobulins (considered Id or anti-Id) would be connected to whole families of not necessarily related Ig's and because of their central location, be considered as regulatory (anti-)ideotypes. Others having few links through the network are more likely to be regulated than regulators. Such a regulatory mechanism certainly makes for a flexible and self-righting system, which can readily adjust and re-equilibrate following a disturbance, *e.g.* foreign antigen. However, it also provides some possible explanations for the origins of autoantibodies. This is qualified as "possible" for the simple reason that, as an interlocking network, one may hypothesize any number of routes to get from one point to another, yet it is extremely difficult to prove which Id initiated a response and which followed. Nevertheless, consistent with this model, many experimental anti-Id behave as organ-specific AAb.

One example concerns the induction of autoantibodies against the acetylcholine receptor (AcChoR) via an anti-idiotype scheme. Erlanger and colleagues immunized rabbits with BisQ, a potent synthetic agonist for the AcChoR. The resulting anti-BisQ antibodies were purified and in turn used as immunogen to stimulate detectable anti-(anti-bisQ) antibodies. These anti-Id reacted with the AcChoR. Although BisQ is an agonist, 2 of the 3 rabbits producing autoanti-receptor Ab exhibited muscle weakness characteristic of experimental myasthenia.[39] Most anti-idiotypic experiments proceed in two stages, as above. However, by constructing hybridomas using spleen cells from animals immunized with BisQ, this group demonstrated that some of the monoclonal Ab behaved directly as anti-AcChoR AAb, *i.e.* an anti-Id response is part of the normal response to BisQ in the same animal.[40] Similarly, mice immunized with insulin produced both anti-insulin and anti-insulin receptor antibodies; the latter anti-Id autoantibodies behaved as receptor-stimulators.[41] Immunization with affinity-purified anti-Id antibody (Ab_2) raised against a monoclonal anti-thyroglobulin (Ab_1), gave several hybridomas having anti-thyroglobulin activity (Ab_3).[42]

In principle, the response to any antigen could trigger an anti-Id or cross-reacting Id (CRI) several links away through the network, and such an immunoglobulin could operationally behave as an autoantibody. Thus an Id (Ab_1) produced in response to Ag would be operationally anti-Id for a series of clones expressing Ab_2, Ab_2', Ab_2'', *etc.*, and these in turn be linked to still additional specificities Ab_3, Ab_3', *etc.*--any of which could be a central regulatory Id and any could react with an autoantigen. It has been suggested that the background or natural antibodies produced in the absence of exposure to external antigen have a high degree of reactivity with other Ids or high "connectivity".[5,6,43] Many of these Ab using conserved Id also exhibit autoantibody activity. That such autoreactive clones are contained within the highly connected population may be important in ensuring proper regulation, but also in maintaining some expression perhaps stimulated by autoreactive T cells and anti-Id. This low level stimulation, proliferation and consequent somatic mutation may be crucial in maintaining useful diversity and divergence from the germ line sequence in these lines.[44]

In many autoimmune diseases,

prominent CRI are apparent. Expression of a single idiotypic family identified by an anti-Id to an anti-DNA monoclonal AAb, correlated better with disease activity than anti-DNA levels despite the fact that many were Id positive, DNA (Ag) negative.[45] Some of these antigen--negative immunoglobulins of the same Id-family, may represent autoantigens co-expressed and co-regulated in the various diseases. Pisetsky *et al.* found that an Id-specific antiserum raised against a monoclonal anti-Sm autoantibody derived from spontaneous autoimmune MRL mice, reacted with another independently-derived anti-Sm, but also with 2 anti-DNA monoclonal Ab.[46] Moreover, the anti-Sm itself possessed anti-DNA reactivity. In both human and murine SLE, anti-Sm and anti-DNA autoantibodies are usually co-expressed; CRIs as well as related antigenic specificities may provide an explanation for some linked sets of AAb.

Plotz has suggested that many autoantibodies actually represent anti-Ids to anti-viral antibodies.[47] Some may behave as internal images and enhance the anti-viral response in a third wave, but to the extent that these second antibodies mimic the antigen they may also behave as autoantibodies binding to (reactive against) precisely the same host components with which the original viral antigen would bind or associate. Such a scheme might be effective to the extent that the first antibodies (Id or Ab_1) are directed against actual sites of interaction and not elsewhere on the same molecule, and to the extent that the second wave does not diverge or cease to mimic the original antigenic site and hence retains reactivity with viral-complementary host structures. Exactly how many of these anti-Id (Ab_2) autoantibodies would be required to evidence an autoimmune disease again might depend upon the normal function of the autoantigen, but in principle, one might suffice. In relation to known autoantigens, it is again relevant that many are structures that might be expected to interact with the intact viruses, viral nucleic acids, *etc.* as discussed in the context of altered-self. Organ-or cell-specific viruses likely utilize hormone or other specific receptors in host cell recognition and attachment, and specific cell surface proteins are frequent targets of AAb.

Since viruses and viral substructures generally interact with host components over at least somewhat restricted sites, and the structural correlate of Id may represent but a few amino acids, the related questions arise as to over what sort of area determinants on AAg could be spread?, would not many AAb be mutually exclusive rather than cooperating in precipitation?, and should epitopes not be clustered toward the virus-interacting "face" of the autoantigen? Insufficient is known about individual epitopes on autoantigens and their complexities to respond in this context at present. Note that one would not anticipate that the resulting autoantibodies would react with original viral antigen since they are related, but "in phase", but an appropriate anti-Id to an AAb might identify the viral antigen.

A plausible case for such a mechanism can be made, however, it is difficult to trace such a route through the network and conclude that it actually is working in a particular disease. A first autoantibody recognizing an altered host structure (Ab_2) could also work in reverse through the network to give Ab_1. Nonetheless, in experimental systems, where we have known reference or start points, and known immunogens, it is possible to work our way through this labrynth in exactly the manner predicted. Thus, starting with mouse antiserum to reovirus which had been absorbed to render it serotype-specific for the hemagglutinin capsid protein, Greene and co-workers produced a rabbit anti-Id with the same lymphoid and neural cell-specificity as the viral serotype, *i.e.,* against the presumed receptor through which the virus attaches and infects.[48] Subsequently they demonstrated that monoclonal antibodies against the idiotype of a reovirus-neutralizing monoclonal antibody similarly identify the common host cell structure used in viral attachment and block viral attachment to neural host cells, but more significantly, sufficiently mimick the hemagglutinin structure to directly elicit reovirus-specific DTH and CTL.[49] Where the emergence of anti-Id might be a serious obstacle to therapy with even human monoclonal antibodies, on the practical side, the possibility of vaccinating with Ab_2 to elicit Ab_3 with reactivity similar to a neutralizing/inactivating Ab_1, is being actively pursued in reference to viruses, bacteria, parasites, tumors, *etc.*[50-54]

Roles of Id/anti-Id and Id-specific cell networks in the maintenance of autoimmunity

A remaining question is how autoantigen-specific B cell clones escape the self-balancing network of Id and anti-Id and gain ascendancy to the point of an autoimmune disease. From the above, one could postulate an enhancing role of anti-Id as surrogate antigen, but this again should fall eventually under regulation. As a metaphor, the term "network" is perhaps particularly appropriate in that if a net is stretched too far it is torn or permanently distorted. Once accomplished, the rest of the network regains its normal shape with perhaps a hole or two, but more or less functions as expected. What got through the hole in the meanwhile is subsequently beyond restraint. Those clones having specificities which would normally

check the proliferation of the escaped clone through the network, now find their own proliferation checked by the rest of the intact network. A new balance or equilibrium of idiotypes and anti-idiotypes is established excluding the few clones now outside the network. The specificities of the clones that escaped and now proliferate unchecked may be irrelevant or may contribute to the "disease", but they probably are related at least temporally to the agent which stretched the net.

An overproduction of a single or small subset of Id could be effected by many means, and perhaps H-chain gene transgenic mice are in some ways analogous. Imanishi-Kari found that such mice spontaneously express Id, but of relevance here, the Id^+ Ig are not encoded by the foreign gene. Furthermore, the Id distortion of the expressed repertoire could be transferred to other mice via T cells.[55] This may or may not pertain to AAb, however, it suggests a mechanism by which a single specificity having at one time escaped the net could become self-perpetuating and actually recruit additional AAb.

Autoantibody sets and cross reactions

A great deal of current research, especially in the rheumatic diseases has concentrated on the autoantibody specificities as central to the various diseases. A basic assumption is that through a better understanding of the AAb, the common thread could be retraced in both directions--back to the origin of the antibodies, the immunogen if any, and further to the etiology of the very disease itself, and in the other direction, to the true target autoantigen involved in pathogenesis. A byproduct of such research has been the integration of structural/functional information on the same entities gained from disparate approaches: autoantigens as central to and implicated in the disease process and autoantigens as normal constituents of cells. The use of certain AAB (*e.g.* those reactive with Sm, RNP, La/Ro, *etc.*) as probes of structure/function has had tremendous impact in our understanding of basic cellular biology at the molecular and biochemical level (the processing of Pol II and Pol III RNA transcripts in the above examples). In return many of these autoantigens are beginning to be dissected at the subunit and even epitope levels. However, one consequence of this apparently profitable approach is that it underscores our confidence that the obvious autoantigen recognized by a particular AAb is the sole autoantigen, with reasonably direct links to the immunogen and pathogenicity. From the various models discussed above it should be apparent that retracing the thread from AAb to immunogens is difficult in the spontaneous diseases. In some diseases there are many parallel threads, some ending in an innocuous AAb and others progressing further to pathogenetic involvement; the threads may or may not unite at the point of a single immunogen or etiological agent.

Many of the above models invoke foreign agents, viruses, microbes, drugs, toxins, *etc.*, as eliciting Ab either directly or via modification of self components, which operationally behave as AAb through crossreaction. This already confusing thread may subsequently be further obscured through an Id/anti-Id cascade or parallel sets of CRI. However, we have only lightly touched on the question of internal crossreaction, where an immunoglobulin, initiated by whatever means, exhibits reactivity with several self components. This even further complicates the identification of the immunogen and has extremely important implications in terms of the pathenogenetic involvement of autoantibody subsets. The best-characterized example pertains to anti-DNA autoantibodies, but the likelyhood is, that the phenomenon is far more general.

As a family, anti-DNA Ab represent the best characterized of all AAb-AAg systems, with the largest library of single *reactivities* captured as monoclonal Ab. Anti-DNA antibodies exhibit an extremely high degree of multispecificity--reaction with related and unrelated structures. Many of the monoclonal anti-DNA AAb react with cardiolipin,[56] a phospholipid which like DNA, posseses phosphodiester linkages. Some react with cytoskeletal proteins, especially vimentin,[57] a phosphorylated, helical intermediate filament. Crossreaction with repeating polyanionic structures, proteoglycans (heparin-SO_4, chondroitin-SO_4) and glycosaminoglycans (hyaluronic acid), has also been reported.[58,59] The above cross-reacting macromolecules have at least some similarity with the repeating structure of DNA and such similarity undoubtedly reflects crucial elements in Ag recognition. Other crossreactions with apparently unrelated structures cannot be explained as easily: that with a surface component of platelets (thrombocytopenia is occasionally associated with SLE),[60,61] with IgG or with the Sm small ribonucleoprotein complex.[46,60] The crossreaction with Sm is of particular significance since both anti-Sm and Anti-DNA are markers is SLE, but until more monoclonal AAb are produced and characterized, is difficult to assess the extent to which multispecificity contributes to the apparent heterogeneity and overlapping sets of co-expressed AAb in rheumatic diseases. A corollary of multispecificity might be that the truely important autoantigens have been overlooked or mis-identified in far more autoimmune diseases. Multispecificity (or common antigens, insofar as actual antigens have not yet been identified) for example, could account for multiple-

organ reactive monoclonal autoantibodies in reovirus-induced autoimmunity.[62]

Multispecificity must be considered in any model of the origin of anti-DNA AAb which implicates an immunogen. This problem can, of course be avoided altogether, by invoking non-specific mechanisms such as polyclonal activation, however doing so does not account for the polyclonality and fact that such a high proportion of the diverse AAb all react with DNA. Is DNA itself the immunogen? Animals immunized with ssDNA, helical synthetic polynucleotides, Z-DNA, *etc.*, do respond but with specificity restricted to the immunizing polynucleotide.[63] The AAb specific for native DNA, diagnostic for SLE, but confined to few sera, are not elicited by immunization of normal or autoimmune (SLE) mice. The elicited Ab typically exhibit minimal crossreaction with cardiolipin, unlike the polyspecific AAb of the spontaneous disease. Cardiolipin, on the other hand, elicits antibodies which react more strongly with denatured DNA than with the immunogen.[64] Thus, in terms of eliciting polyspecific anti-DNA Ab characteristic of the disease, cardiolipin seems to more closely correspond to the immunogen than DNA. Surprisingly, lupus mice which spontaneously produce antibodies to dsDNA, ssDNA and cardiolipin failed to respond to these as immunogens.[63] Thus, the spontaneous AAb appear to be derived from different clones, and assigning an immunogen is not so simple. On the other hand, crossreaction with cardiolipin applies to at most one-third of the anti-DNA AAb. Furthermore, the anti-DNA AAb are polyclonal and the one common antigen of this heterogeneous mix, regardless of multispecificity and preference, is DNA--not cardiolipin. Harding has recently reminded us that the anti-histone response in SLE is second only to the anti-DNA response. He suggests that in so far as these macromolecules are naturally associated *in vivo,* a DNA-histone complex might serve as immunogen,[65] particularly in light of the repeated observation that associated protein vastly improves the immunogenicity of polynucleotides.

Anti-DNA Ab, implicated in immune complex glomerulonephritis are the only SLE AAb with well-established roles in pathogenesis, however, does this mean that they exert their pathogenetic effects through reaction with DNA? Eilat has suggested that the multispecificity of these AAb calls for a reinvestigation of the evidence for DNA immune complexes in the kidney, as it is largely based upon these AAb used in immunofluorescence. Little free circulating DNA exists and immune complex clearance by the reticulo-endothelial system appears unimpaired in SLE.[59] Rather, he suggests, a fixed antigen such as a proteoglycan in the glomerular basement membrane could account for the deposition of anti-DNA autoantibodies and resulting pathological changes. The involvement could be even more pervasive; a single anti-DNA monoclonal antibody was recently reported to recognize the same or similar proteins in glomeruli, platelets, erythrocytes, T- and B-lymphoblast cell lines and neuronal membranes.[66] How many other AAb have been assigned to but a single apparent or abundant AAg, thus overlooking their true significance in the associated disease?

Summary

Cells with receptors capable of binding self-antigens constitute a normal component of the B cell repertoire; these specificities appear to be represented in low levels within the expressed antibody population. In autoimmune disease, this potential-actual repertoire is skewed in favor of exaggerated autoantibody expression. The clones or specificities affected are characteristic, even diagnostic of the particular disease, yet individuals express but a relatively small subset of this complexity. "Marker" autoantibodies may or may not be included, but autoantibodies more typical of other, related diseases are usually present. Certain specificities are clearly implicated in the pathogenesis of the corresponding disease; others contribute to- or exacerbate pre-existing pathological conditions, and still others appear at present as epiphenomena. Organ-/tissue-specific autoantibodies largely fall into the first or perhaps second categories; those associated with the systemic rheumatic diseases fit better into the latter or latter two categories. As we learn eventually more about these antibodies and the full spectra of autoantigens with which they react, many more may be found to exert more central roles in pathogenesis. The distortion of the expressed repertoire represents but one aspect of a far more complex set of pre-dispositions, conditions, stimuli, contributory factors and perhaps coincidences involved in the onset and perpetuation of the disease. The AAb arise within the context of a disease more pervasive than just the autoantibodies themselves, however, in certain diseases the subsequent autoimmune response may surpass all other variables to constitute the single most clinically significant factor.

In attempting to account for these individual specificities and their disease associations, certain models seem applicable to selected autoantibodies but not to others. Even in these selected cases, the models remain speculative; in no case can we categorically state how the specificities arose. Non-specific activation may contribute to- and expand the expressed autoantibodies,

but such mechansisms generally fall short in terms of adequately explaining the specificities of autoantibodies, the overlapping sets, the polyclonality of the response against single autoantigens or the specific disease associations. Despite this theoretical criticism, empirically, non-specific mechanisms have given rise to mixtures of autoantibodies which appear identical at least in terms of specificities to their spontaneous counterparts in various rheumatic diseases. Most models for the origin of autoantibodies involve immunogens, but they differ in terms of how related the autoimmunogen is to the autoantigen and how direct is this connection. On the one hand, the immunogen may represent a surface antigen altered by association with a drug, toxin, virus, viral component, *etc.*, or simply present on a cell inappropriately expressing MHC-II genes, or any cellular constituent released from injured cells in conjunction with bacterial mitogens/activating factors. Yet the immunogen need not bear any obvious structural/functional relationship with the apparent autoantigen and the latter may not be the crucial target(s); pathogenic involvement, if any, may exist at the level of but a single epitope. Retracing the thread from obvious specificities to pathogenetic specificities in one direction and to eliciting immunogens in the other is further complicated by multispecificities, crossreacting- or regulatory idiotypes and idiotype, anti-idiotype interactions. The thread(s) must exist, but we are just beginning to understand some of the inter-relationships between immunogens, autoantigens and autoantibodies and between various co-expressed autoantibodies and the diseases with which they are associated.

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