

13th FORUM
IN IMMUNOLOGY**THE ORIGIN AND NATURE
OF AUTOANTIBODIES****INTRODUCTION**by **M. Seligmann**

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There is still much ignorance and controversy about the origin and nature of autoantibodies. It was therefore of interest to ask some of those who have recently contributed new data in the field to summarize their views on this problem.

One of the main unsolved questions is the nature of the immunogen (if any) that triggers the production of autoantibodies. Many believe that it is not the autoreactive antigen, and one of the most frequently studied models is that of anti-DNA antibodies. Whether the autoreactive B cells are triggered by internal or environmental antigen is open to question. The wide spectrum of cross-reactivity of monoclonal anti-DNA antibodies includes, on one hand, autoantigens such as endogeneous phospholipids (R. Schwartz) and cell membrane proteins (J.F. Bach), and on the other hand, bacterial polysaccharides (R. Schwartz). The development of anti-DNA antibodies in experimental protocols such as the induction of neonatal tolerance may reflect the combination of such antigenic trigger and polyclonal B-cell activation (P.H. Lambert). It

should be noted at this point that the situation may be different for autoantibodies directed to native or to denatured DNA.

In view of the close relationship between some autoantibodies and the so-called natural antibodies which, although polyspecific, may display high affinity (S. Avrameas), one may wonder whether some autoreactive B cells are triggered by non-specific signals without being antigen-driven, at least in a first stage. This hypothesis would imply that the production of such autoantibodies is an inherent property of the normal immune system. Several laboratories have recently provided evidence that some autoantibodies are indeed encoded by restricted sets of germ-line V genes. There is suggestive evidence that naturally occurring autoreactive antibodies are produced in mice by B cells belonging to the Ly1-B lineage (L.A. Herzenberg).

The results reported by R. Schwartz showing that immunization with a hapten leads, as the V_H gene mutates, to the loss of the anti-DNA reactivity of

the pre-immune IgM antibodies and to acquisition of anti-hapten activity, are apparently in contradiction with those, described by B. Diamond, suggesting that anti-DNA autoantibodies (belonging to the IgG class) may be generated by somatic mutation of germ-line genes encoding «natural» antibacterial antibodies. In this context, it should be noted that, although Avrameas has found anti-actin activity of several IgG and IgA myeloma proteins, the incidence of various autoantibody activities, such as cold agglutinins, rheumatoid factors, etc., is extremely high among human monoclonal immunoglobulins belonging to the IgM class. It should also be pointed out that a close relationship between the «natural»

autoantibodies and the pathogenic autoantibodies of autoimmune diseases is far from proven.

Finally, most of the above-mentioned hypotheses on the production of autoantibodies are difficult to reconcile with the importance of autoantigen presentation by epithelial cells expressing HLA class II molecules in the pathogenesis of autoimmune thyroid diseases, as outlined by G. Bottazzo and M. Feldman. Such a mechanism may apply only to organ-specific autoimmunity.

The discrepant views expressed by the contributors to this forum suggest that the nature and origin of autoantibodies are not univocal.

NATURAL AUTOREACTIVE B CELLS AND AUTOANTIBODIES: THE «KNOW THYSELF» OF THE IMMUNE SYSTEM

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*«To know thyself is useful everywhere»
Socrates*

INTRODUCTION

From its early beginning, a rather finalistic role has been attributed to the immune system; the antibodies were there in order to protect the organism from foreign invasions. In this rather military defence concept, antibodies able to react with self constituents were considered not only unnecessary but harmful because, if they were generated, they would lead, as stated by P. Ehrlich, to a «horror autotoxicus» and destruction of the organism [11]. Such a view, that the immune system is exclusively directed against the external

foreign environmental antigens, has been further strengthened by the classical studies of Landsteiner showing the extremely high specificity of the antibodies [22]. Substitution of one halogen by another in a small molecular weight constituent resulted in the non-recognition of the thusly modified constituent by the antibody. The spectacular success encountered by vaccines further established this point of view [23]. The clonal selection theory, as formulated by Burnet, conceptualized and put into a clear theoretical framework

these series of experiments and results [5]. According to this theory, each lymphocyte possesses an antibody receptor strictly specific for a foreign antigen; clones capable of reacting with self components are deleted during foetal life.

There is no doubt that a long series of experiments has given clear-cut results supporting the above conclusion. At the same time, however, at least in my mind, there is no doubt that the vast majority of experimental designs which gave rise to these results were far removed from the reality usually encountered by the immune system in physiopathological situations. Injections into animals of high amounts of protein antigens incorporated into various adjuvants are needed in order to obtain detectable levels of antibodies, and similarly, injections of millions of erythrocytes are required in order to induce detectable plaque-forming cells. Therefore, one has to pose the question as to whether the results obtained under these experimental conditions, although real and irrefutable, correspond to only a partial aspect of the immune system not necessarily linked with its normal physiopathological functioning. Indications that this might be the case could have been deduced from the early studies reporting the presence in sera of physiologically healthy individuals and animals, of antibodies, subsequently termed natural antibodies, often reacting with self antigens [3, 21, 4]. Although, until the early fifties, a great deal of work was devoted to natural antibodies, during recent years only occasional studies on this subject have been reported. Until very recently, natural antibodies were considered as rather curious but uninteresting, and probably not a serious aspect of the immune system; it is worth noting that only rarely have investigators been interested in the possible immunophysiopathological role of natural antibodies [4, 18, 13, 14, 26, 2, 6].

In our laboratory in 1977, while attempting to prepare a monospecific anti-tubulin antiserum, we noted that in fact anti-tubulin antibodies were present in the sera of various animal species before any experimental immunization

[19]. This initial observation was followed by a series of experiments aimed at studying the natural antibodies and examining their relationships with the autoantibodies. Using a panel of self and non-self antigens (albumin, actin, DNA, myoglobin, myosin, peroxidase, renin, spectrin, thyroglobulin, TNP-protein conjugates and transferrin), we found that autoreactive B-cell clones and natural autoantibodies were present in humans [1, 15, 7, 8], mice [9, 10] and rats [25]. A few natural autoantibodies were found to be monospecific, *i.e.* to react with only one antigen on the panel. The vast majority of the natural autoantibodies, however, whether polyclonal or monoclonal, were found to be polyspecific, *i.e.* to react with more than two self and non-self antigens on the panel. Thus, murine monoclonal natural antibodies reacted with such dissimilar antigens as DNA, myosin, thyroglobulin, spectrin and TNP [9]. Cross-reactive idiotopes were found frequently among the murine monoclonal polyspecific natural autoantibodies and were also present in normal mouse sera associated with certain immunoglobulin subclasses [24].

More recent studies on this subject have included investigations of the cross-reactive idiotopes of natural autoantibodies among humans, mice and rats, the affinity of natural autoantibodies and their presence and specificities in mice at different stages of the immune response, as well as studies on the capacity of mice to respond to injections of self antigens.

Cross-reactive idiotopes of natural autoantibodies.

Monoclonal immunoglobulins secreted by hybrids derived from the fusion of either normal human peripheral lymphocytes with a non-secreting murine myeloma cell line (Blancher *et al.*, unpublished results) or spleen cells from BN rats with a non-secreting rat myeloma cell line [25] were examined for antibody activity using the panel of antigens described above. Monoclonal immunoglobulins with polyspecific

natural autoantibody activity were found, as well as monospecific antibodies and antibodies with restricted polyspecificity, that is, reacting with only two to three antigens on the panel. Using a rabbit antiidiotypic antibody raised against a BALB/c monoclonal polyspecific natural autoantibody, we analysed the cross-reactive idiotypic determinants of these antibodies. We noted that a substantial part of human and all rat autoantibodies which possessed polyspecific reactivities bore the murine idiotopes. With a few exceptions observed with human antibodies, monospecific antibodies were not found to carry the murine idiotopes. In this connection, it is interesting to note that between the two rat monoclonal IgE examined, one monospecific for TNP and the other possessing polyspecific reactivities, only the latter was found to bear the cross-reactive idiotopes [25].

These findings, as well as those already obtained with mice, show that polyspecific natural autoantibodies correspond to highly conserved recurrent and frequent idiotopes. Furthermore, they strongly suggest that restricted families of germline genes may encode for at least some of the polyspecific natural autoantibodies among humans, mice and rats.

Affinity of Natural Autoantibodies.

The fact that polyspecific natural autoantibodies are able to react with various dissimilar antigens raises the question of the antigenic structure which they are recognizing and, consequently, of their affinity. Using a method published recently by Friguier *et al.* [12], we measured the dissociation constant (K_D) of several polyspecific natural monoclonal autoantibodies for different macromolecular antigens and haptens and we compared the values obtained with those of induced monospecific antibodies produced after active immunization. The results obtained indicate that: 1) the K_D of the monoclonal polyspecific natural autoantibodies for macromolecules ranges between 10^{-5} and 10^{-10} M and, for a given antigen, is often on the same order of

magnitude as that of induced monoclonal or polyclonal antibodies; on this basis, it appears that the generally admitted correlation between antibody specificity and affinity does not always hold true; 2) in contrast with induced monospecific antibodies, the K_D of natural autoantibodies for free hapten are high, whereas they are low when the same hapten is associated with a macromolecule; thus it appears that, although natural and induced autoantibodies exhibit similar «functional affinities» [20], that is, avidities for a macromolecule, they differ in their «intrinsic affinities» [20] for a given hapten or epitope; and 3) although the monoclonal polyspecific natural antibodies examined exhibit similar broad reactivities for several antigens, their fine specificities for these antigens, as defined by the measurement of their K_D , are different; thus, it appears that each monoclonal polyspecific natural antibody can be considered unique [28].

Natural autoantibodies in mice at different stages of the immune response.

Mice were injected with various antigens (human serum albumin, rat myosin, histamine-ovalbumin conjugate, 5-bromuridine-bovine serum albumin conjugate) in complete Freund's adjuvant following various injection schedules. Hybridomas were produced from the spleen of these immunized mice and examined for their production of antibodies directed against the antigen injected and against a panel of self (tubulin, actin, myosin, DNA) and non-self antigens (whale myoglobin, human spectrin, horseradish peroxidase, trinitrobenzene coupled to bovine serum albumin). In addition to a high number of hybrids synthesizing monospecific antibodies against the immunizing antigen, 2-5 % of the hybrids were found to secrete polyspecific natural autoantibodies. Furthermore, several hybrids were also found to secrete antibodies reacting with the immunizing antigen as well as one or more antigens on the panel [16].

Similarly, murine monoclonal anti-H₂ [27], anti-HLA-DR [17] or polio virus neutralizing antibodies (R. Gon-

zalez *et al.*, unpublished results) obtained after fusion of splenocytes from mice immunized with the corresponding antigens, were found to react with one or more self antigens.

The above results support our previously advanced hypothesis [9, 16] that cells carrying polyspecific natural autoantibodies as receptors after antigenic stimulation proliferate into cells producing antibodies more and more specific for the immunizing antigen. In this context, it is interesting to note that we have found that hybrids derived from the spleens of old BALB/c normal mice or from 4-month old MRL mice with a lupus-like autoimmunity syndrome secrete monoclonal autoantibodies, for which the great majority were monospecific, as compared to the polyspecific natural autoantibodies (W. Mahana *et al.*, unpublished results).

Response of mice to injections of self antigens.

It is widely accepted that animals do not respond to self antigens because of a presumed elimination and/or anergy of autoreactive cell clones. This broadly admitted hypothesis is somehow in contradiction with the studies on naturally occurring autoantibodies which have demonstrated that the humoral immune systems of humans, mice and rats are able to recognize self antigens. In order to test whether the immune system is able to respond to self stimulation, newborn or adult BALB/c mice were injected repeatedly with either syngeneic (BALB/c) or xenogeneic (bovine) proteins (myosin, albumin, actin) or with syngeneic or xenogeneic albumin coupled with TNP, dissolved in physiological saline, and the presence of anti-protein or anti-TNP antibodies in the sera of immunized mice was then evaluated. The results obtained indicate that newborn but not adult mice are capable of responding to self stimulation. These results also suggest that one of the primary functions of the immune system in newborn mice is the recognition of self (submitted for publication).

Conclusions and hypotheses.

The whole of the results we have obtained with natural autoantibodies strongly suggests that the recognition of self antigens, mainly through polyspecific receptors able to react with both self and non-self antigens, constitutes one of the basic rules of the immune system. In such a concept, and in accordance with the results obtained, one would expect that the polyspecific autoreactive B-cell clones and their synthesized products, the polyspecific autoantibodies, would be of germline origin and would be present in an organism for the normal physiological functioning of the immune system.

Autoreactive B cells recognize the self antigen through the polyspecific autoantibody receptor, as such; and perhaps, precisely because the receptor fits perfectly with the self antigen, the cells are minimally stimulated, if at all. Therefore, this kind of equilibrium cannot be broken by self antigens unless (as in the experiments we have performed with newborn mice), because of repeated injections, much higher amounts of self antigens than normal are present at a time when the regulatory system has not yet been well established. In contrast, sufficient accumulation of a non-self antigen will be able to break this equilibrium, because recognition by the polyspecific receptor of the antigen is imperfect and it is recognized as non-self. It is evident that this equilibrium can also be broken when the self antigen, for any reason, for example, by infections or use of adjuvants, has been sufficiently altered so as to be recognized as non-self.

In these cases, cells carrying the polyspecific natural autoantibodies as receptors will be stimulated and proliferate into cells producing more and more specific antibodies for the non-self antigen. This hypothesis is supported by results obtained with the hybrids derived from the immunized mice, whose monoclonal antibodies were found to react either with the immunizing antigen alone, with the immunizing antigen plus one antigen, or with two, three or more self and non-self antigens. The

molecular mechanisms that could, through proliferation, lead from cells carrying polyspecific autoantibody receptors to cells synthesizing and secreting monospecific antibodies are not evident. Because, in the mouse (but not in man), polyspecific natural autoantibodies are often found to be associated with the IgM isotype and only rarely with IgG, it would appear that, at least in mice, these mechanisms are related to the switch from IgM to IgG. It is possible that mutational events contribute to these modifications from polyspecificity to monospecificity. However, taking into account that polyspecific natural autoantibodies possess affinities on the same order of magnitude as monospecific induced antibodies, one would expect to find here, as well, a still unknown mechanism involving gene rearrangements.

It is evident that, in an adult organism, all situations, from cells producing polyspecific natural autoantibodies to cells synthesizing monospecific antibodies against non-self antigens, may exist. The relative importance of these situations will depend upon the animal's immunological history. It is conceivable that, due to various stimuli, for example, viral, oncogenic or mitogenic, the few cells that at each time produce antibodies of given specificities can be induced to expand clonally. On this basis, an immunopathological situation involving the presence of relatively high amounts of antibodies can be considered as corresponding to the magnified picture of a given immunophysiological situation which normally occurs, among many others. In an immunopathological situation, however, one would expect that monospecific autoantibodies would

be more harmful than polyspecific ones. This would be so not because their affinity and specificity are higher than those of polyspecific autoantibodies, but rather because they can be inhibited from binding to the target antigen by only one given antigen, whereas the polyspecific antibodies can be blocked by many cross-reacting antigens.

Finally, one would expect that, in foetal life or soon after birth, depending upon the animal species, the immune system, through polyspecific receptors, would mainly recognize internal self antigens. This behaviour of the immune system is consistent with a general rule of ontogenesis and organogenesis which is primarily the recognition of self specificities without which a proper assembly of the organism is impossible. In contrast, one would expect that, in an old animal, as in an experimentally immunized adult animal, mainly monospecific antibodies often directed against non-self antigens would be present. The difference between immunized and old animals is that cells synthesizing polyspecific natural antibodies would be only marginally generated in old animals, if at all.

In summary, I consider that, through polyspecific receptors, the immune system is able to recognize self internal antigens and that this recognition constitutes the basis for the immune system being able to recognize and respond to non-self external antigens. Thus, the immune system, like the other biological systems, like the various animal species, like human society or even like philosophy, has to possess the «know thyself».

References.

- [1] AVRAMEAS, S., GUILBERT, B. & DIGHERO, G., Natural antibodies against tubulin, actin, myoglobin, thyroglobulin, fetuin, albumin and transferrin are present in normal human sera and monoclonal immunoglobulins from multiple myeloma and Waldenström's macroglobulinemia may express similar antibody specificities. *Ann. Immunol. (Inst. Pasteur)*, 1981, 132 C, 231-236.

- [2] AVRAMEAS, S., DIGHIERO, G., LYMBERI, P. & GUILBERT, B., Studies on natural antibodies and autoantibodies. *Ann. Immunol. (Inst. Pasteur)*, 1983, **134 D**, 103-113.
- [3] BESREDKA, M., Les antihémolysines naturelles. *Ann. Inst. Pasteur*, 1901, **15**, 785-807.
- [4] BOYDEN, S.V., Natural antibodies and the immune response. *Advanc. Immunol.*, 1963, **5**, 1-28.
- [5] BURNET, F.M., The clonal selection theory of acquired immunity. Cambridge University Press, London, New York, 1959.
- [6] COUTINHO, A., FORNI, L., HOLMBERG, D., IVARS, F. & VAZ, N., From an antigen-centered, clonal perspective of immune responses to an organism-centered, network perspective of autonomous activity in a self-referential immune system. *Immunol. Rev.*, 1984, **79**, 151-168.
- [7] DIGHIERO, G., GUILBERT, B. & AVRAMEAS, S., Naturally occurring antibodies against nine common antigens in human sera. — II. High incidence of monoclonal Ig exhibiting antibody activity against actin and tubulin and sharing antibody specificities with natural antibodies. *J. Immunol.*, 1982, **128**, 2788-2792.
- [8] DIGHIERO, G., GUILBERT, B., FERMAND, J.P., LYMBERI, P., DANON, F. & AVRAMEAS, S., Thirty-six human monoclonal immunoglobulins with antibody activity against cytoskeleton proteins, thyroglobulin, and native DNA: immunologic studies and clinical correlations. *Blood*, 1983, **62**, 264-270.
- [9] DIGHIERO, G., LYMBERI, P., MAZIE, J.C., ROUYRE, S., BUTLER-BROWNE, G.S., WAHLEN, R.G. & AVRAMEAS, S., Murine hybridomas secreting natural monoclonal antibodies reacting with self antigens. *J. Immunol.*, 1983, **131**, 2267-2772.
- [10] DIGHIERO, G., LYMBERI, P., HOLMBERG, D., LUNDQUIST, I., COUTINHO, A. & AVRAMEAS, S., High frequency of natural autoantibodies in normal newborn mice. *J. Immunol.*, 1985, **134**, 765-771.
- [11] EHRLICH, P. & MORGENROTH, J., Ueber Haemolysine. *Berl. klin. Wschr.*, 1901, **21**, 569-574.
- [12] FRIGUET, B., CHAFFOTE, A.F., DJAVADI-OHANIANCE, L. & GOLDBERG, M., Measurements of the true affinity constant in solution of antigen-antibody complexes by enzyme-linked immunosorbent assay. *J. Immunol. Methods*, 1985, **77**, 305-319.
- [13] GRABAR, P., Autoantibodies and immunological theories: an analytic review. *Clin. Immunol. Immunopath.*, 1975, **4**, 453-466.
- [14] GRABAR, P., Autoantibodies and the physiological role of immunoglobulins. *Immunol. Today*, 1983, **4**, 337-340.
- [15] GUILBERT, B., DIGHIERO, G. & AVRAMEAS, S., Naturally occurring antibodies against nine common antigens in human sera. — I. Detection, isolation, and characterization. *J. Immunol.*, 1982, **128**, 2779-2787.
- [16] GUILBERT, B., MAHANA, W., GILBERT, M., MAZIE, J.C. & AVRAMEAS, S., Presence of natural autoantibodies in hyperimmunized mice. *Immunology*, 1985, **56**, 401-408.
- [17] GUILBERT, B., FELLOUS, M. & AVRAMEAS, S., Anti-HLA-DR monoclonal antibodies cross-react with several self and non-self non-MHC molecules. *Immunogenetics*, 1986 (in press).
- [18] JERNE, N.K., The natural selection theory of antibody formation. *Proc. nat. Acad. Sci. (Wash.)*, 1955, **41**, 849-857.
- [19] KARSENTI, E., GUILBERT, B., BORNENS, M. & AVRAMEAS, S., Antibodies to tubulin in normal non-immunized animals. *Proc. nat. Acad. Sci. (Wash.)*, 1977, **74**, 3997-4001.
- [20] KARUSH, F., Multivalent binding and functional affinity. *Contempor. Top. Mol. Immunol.*, 1976, **5**, 217-228.
- [21] KENNEDY, W.P., The production of spermatoxins. *Quart. J. exp. Physiol.*, 1924, **14**, 279-283.

- [22] LANDSTEINER, K., The specificity of serological reactions. Harvard University Press, Cambridge, Massachusetts, 1945.
- [23] LERNER, R.A., CHANOCK, R.M. & BROWN, F., Vaccines 85. Cold Spring Harbor Laboratory, New York, 1985.
- [24] LYMBERI, P., DIGHERO, G., TERNYNCK, T. & AVRAMEAS, S., A high incidence of cross-reactive idiotypes among murine natural autoantibodies. *Europ. J. Immunol.*, 1985, **15**, 702-707.
- [25] LYMBERI, P., HIRSCH, F., KUHN, J., TERNYNCK, T., DRUET, P. & AVRAMEAS, S., Autoimmunity induced by HgCl₂ in brown-Norway rats. *J. Immunol.*, 1986 (in press).
- [26] MACKAY, I.R., Natural autoantibodies to the fore-forbidden clones to the rear? *Immunol. Today*, 1983, **4**, 340-341.
- [27] PLA, M., ROCCA, A., GUILBERT, B., REBOUL, M., DASTOT, H., COLOMBANI, J. & AVRAMEAS, S., Anti H-2 monoclonal antibody recognizes self cytoskeletal structures. *Immunogenet.*, 1986 (in press).
- [28] TERNYNCK, T., GREGOIRE, J., LYMBERI, P. & AVRAMEAS, S., A study of the dissociation constants of murine polyspecific natural autoantibodies. *Mol. Immunol.*, 1986 (submitted for publication).

ARE NATURAL AUTOANTIBODIES REAL?

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During the past 85 years, ideas about autoimmunity have gone through two distinct stages, which can be referred to as: Ehrlich's *horror autotoxicus* and Burnet's forbidden clones. Ehrlich's own experimental evidence contradicted the very idea of autoimmunity, and he concluded that such a process would be improbable. By Burnet's time, 50 years after Ehrlich, many examples of autoimmune diseases had been uncovered. Therefore, he had a different perspective: autoimmunity is indeed possible, but it is an aberration. Now we have entered a third phase in the evolution of ideas about autoimmunity. The emerging new concept is that the ability to form autoantibodies is an inherent property of the normal immune system. In other words, autoreactivity is not purged during ontogeny, but preserved. It is difficult to predict how long this third concept will last, but it seems to be an idea whose time has come. There are, however, formidable problems to solve before we can accept the relevance

of «natural» autoantibodies to either autoimmune diseases or to fundamental mechanisms of the normal immune system.

There are many examples of «natural» autoantibodies. They usually have specificity for highly conserved structures: xanthine oxidase, myelin basic protein, collagen, nucleic acids and cytoplasmic filaments are examples. These kinds of antibodies probably account for the so-called «background» that is encountered in almost all serological tests for autoantibodies. They are present in serum from almost all normal subjects and in serum from all age groups. Antibodies against tubulin, actin, thyroglobulin, myoglobin, fetuin, transferrin, albumin, cytochrome *c* and collagen have been found in pooled serum obtained from a large number of unselected normal humans [1]. Autoantibodies to neural tissue [2] and to sperm proteins [3] also occur in most samples of normal serum.

Dighiero *et al.* [4] found that hybridomas derived from unimmunized 6-day old mice can produce autoantibodies. About 6 % of the hybridomas produced monoclonal antibodies against a panel of nine autoantigens. And in another study, about 12 % of hybridomas derived from normal mice produced antibodies against pancreas, stomach, salivary glands and pituitary [5]. After depletion of T cells, suspensions of splenocytes from normal mice yield a high proportion (30 %) of B-cell-derived hybridomas (Y. Naparstek, unpublished observation). Cairns *et al.* [6] prepared hybridomas from the tonsillar lymphocytes of a normal 7-year old girl: almost one-third of the clones secreted autoantibodies. It is unlikely that such «natural» autoantibodies are the result of stimulation by bacterial antigens because they are found even in germ-free mice [7].

The antigens to which these autoantibodies bind belong to a class of ubiquitous autoantigens: nucleic acids, cytoskeletal proteins, transferrin, albumin and the Fc portion of IgG are examples. Moreover, extensive cross-reactivity is a prominent feature. All, or nearly all, of them are IgM antibodies. Moreover, their counterparts occur in Waldenstrom's macroglobulinaemia, multiple myeloma and benign monoclonal gammopathies [8]. These autoreactive paraproteins are not unusual: of 612 paraproteins, about 6 % bound to a panel of 9 autoantigens, mainly cytoskeletal proteins [9]; in another series, 9 % of 265 monoclonal gammopathies reacted with nucleic acid antigens [10].

Are the reactions of these natural autoantibodies an artefact? Is their binding a function of the variable region or are the observations due to some non-specific «sticky» property of IgM antibodies? Does the extremely sensitive ELISA technic, which has been used for most of the studies just summarized, exaggerate out of proportion irrelevant binding properties of the antibodies? Do the ubiquitous autoantigens to which the antibodies bind, and with which they cross-react extensively, share some banal property that leads to unimportant ionic binding on solid surfaces?

Is there, in other words, a systematic technical error that has misled dozens of investigators?

All these questions are important to answer before the reality of natural autoantibodies can be generally accepted. We have to admit that these issues have not been addressed rigorously in all cases. Nevertheless, some evidence supports the validity of the observations. That the variable region is indeed involved has been shown by a point mutation in the variable region of an immunoglobulin VH gene, resulting in a single amino acid substitution which changed the binding specificity of the «wild type» antibody from anti-phosphocholine to anti-DNA [11]. Moreover, the mutated antibody bound to DNA in a liquid phase filter assay. A related observation is that a chimaeric protein (created by *in vitro* mutagenesis) with a heavy chain V region sequence identical to that of a DNA-binding antibody, except for a single amino acid substitution, did not bind to ssDNA [12]. Another demonstration of the authenticity of the binding reactions with nucleic acid antigens is that they can be specifically inhibited by small haptens [13] or anti-idiotypes in the solution phase [14]. Still, the immunological specificity of the reactions of «natural» autoantibodies is a subject requiring scrupulous attention by all who work in the field.

I will, for the sake of this position paper, assume that natural autoantibodies are indeed real. Moreover, the logic of the immune system virtually demands that they exist. It is becoming evident that the complex organization of the mammalian immune system arose by evolution from a much simpler primordial mechanism. Recent studies of the horned shark (*Heterodontus francisii*), from which mammalian precursors diverged 450 million years ago, have shown that its heavy chain immunoglobulin genes consist of a simple cassette of three genes — V, D and J — linked to a single set of C genes. These genomic clusters, which are repeated many times, form a V-D-J exon by deletion of intervening DNA. The main source of immunoglobulin variation in this

species is brought about by imprecise fusion of the elemental V, D and J regions (junctional diversity) [15]. In mammals, by contrast, there are several hundred different V genes, about two dozen different D genes and four different J genes. The combination of these different genes, together with junctional diversity and somatic mutation, account for the principal sources of antibody variation in higher species [16]. But the extraordinary ability of mammals to generate antibodies against a virtually unlimited number of antigens evolved at a price: the inevitable formation of autoantibodies as a result of recombination, junctional diversity and somatic mutation of V genes. Thus, it seems only «natural» that autoantibodies exist in normal serum.

In this brief paper, I will not deal with the important and complex question of the relationship, if any, of natural autoantibodies to the antibodies of autoimmune diseases. An equally compelling question is why natural autoantibodies are produced. Actually, they may have no «purpose». Natural autoantibodies may simply be the outward signs of V gene rearrangements, junctional imprecisions and random mutations. In other words, the autoantibodies themselves are not «preserved» by evolutionary forces, but the molecular mechanisms that generate immunoglobulin diversity. A second possibility, raised by Grabar [17], is that natural autoantibodies are «transporters of catabolic products»; *i.e.*, they constitute «a physiological mechanism for cleansing the organism by opsonization of undigested macromolecular 'self'» [18]. Grabar's hypothesis is ingenious, but the difficulty in devising a definitive test of its validity has not been overcome.

Now there is another, quite unusual, idea: natural autoantibodies are the precursors of antibodies induced by exogenous antigens [12, 19]. This new concept is based on three lines of evidence. (a) Autoantibodies that bind to ubiquitous autoantigens (*e.g.*, single-stranded

DNA and cytoskeletal proteins) are polyreactive — *i.e.*, they cross-react with numerous, apparently unrelated autoantigens. (b) Anti-DNA antibodies also cross-react with typical exogenous antigens, such as organic chemical haptens and bacterial polysaccharides [20]. (c) After immunization with exogenous antigens, hybridomas were obtained which secreted antibodies against both the immunizing antigen and autoantigens [12, 19]. By means of a probe encoding the VH gene segment of the dominant population of antibodies which A/J mice produce after immunization with arsonate, it was shown that, as that gene mutates during the response to the hapten, the corresponding pre-immune antibodies lose anti-DNA reactivity and gain anti-arsonate activity [12]. This experiment demonstrates directly that germline V genes encode autoantibodies, and *indirectly* that immunization with a hapten leads to a progression of events that begins with autoreactivity and terminates with anti-hapten activity. Presumably, as exogenous antigen enters the system, it binds to and stimulates B cells expressing polyreactive (autoreactive) immunoglobulin surface receptors. Subsequently, selection by antigen and mutation of V genes results in «conversion» of the autoantibodies to anti-hapten antibodies.

Whether or not this interpretation is correct remains to be seen. But the results again raise the key issue of the meaning of the binding reactions with ubiquitous autoantigens. Is the binding to single-stranded DNA merely a coincidence, even if it is immunochemically authentic, with the «real» antigen remaining unknown. Or is the crucial element polyreactivity — the binding to multiple antigens, regardless of origin — which would, of course, greatly magnify the diversity of the immune response? Is the immune system «naturally» primed to the exterior world by virtue of the polyreactivity of its pre-immune receptors? This is a question of fundamental importance to our understanding of how the system works.

References.

- [1] GUILBERT, B., DIGHIERO, G. & AVRAMEAS, S., *J. Immunol.*, 1982, **128**, 2779-2787.
- [2] STEFANSSON, K., MARTON, L.S., DIEPERNIK, L.S. et al., *Science*, 1985, **228**, 1117-1119.
- [3] RODMAN, T.C., LAURENCE, J., PRUSLIN, F.H. et al., *Science*, 1985, **228**, 1211-1215.
- [4] DIGHIERO, G., LYMBERI, P., HOLMBERG, D. et al., *J. Immunol.*, 1985, **134**, 765-771.
- [5] PRABHAKAR, B.S., SAEGUSA, J., ONODERA, T. et al., *J. Immunol.*, 1984, **133**, 2815-2817.
- [6] CAIRNS, E., BLOCK, J. & BELL, D.A., *J. clin. Invest.*, 1984, **74**, 880.
- [7] UNDERWOOD, J.R., PEDERSEN, J.S., CHALMERS, P.J. et al., *Clin. exp. Immunol.*, 1985, **60**, 417-426.
- [8] SELIGMANN, M. & BROUET, J.C., *Sem. Hematol.*, 1973, **10**, 163.
- [9] DIGHIERO, G., GUILBERT, B., FERMAND, J.P. et al., *Blood*, 1983, **62**, 264.
- [10] SHOENFELD, Y., BEN YEHUDA, O., NAPARSTEK, Y. et al., *Blood* (in press).
- [11] DIAMOND, B. & SCHARFF, M.D., *Proc. nat. Acad. Sci. (Wash.)*, 1984, **81**, 5841-5844.
- [12] NAPARSTEK, Y., ANDRE-SCHWARTZ, J., MANSER, T. et al., *J. exp. Med.* (in press).
- [13] NAPARSTEK, Y., DUGGAN, D., SCHATTNER, A. et al., *J. exp. Med.*, 1985, **161**, 1525.
- [14] SHOENFELD, Y., ISENBERG, D.A., RAUCH, J. et al., *J. exp. Med.*, 1983, **158**, 718.
- [15] HINDS, K.R. & LITMAN, G.W., *Nature (Lond.)*, 1986, **320**, 546.
- [16] TONEGAWA, S., *Nature (Lond.)*, 1983, **302**, 575.
- [17] GRABAR, P., *Ann. Inst. Pasteur*, 1970, **118**, 393.
- [18] GRABAR, P., *Immunol. Today*, 1983, **4**, 337.
- [19] GUILBERT, B., MAHANA, W., GILBERT, M. et al., *Immunology*, 1985, **56**, 401.
- [20] SERBAN, D., RORDORF-ADAM, C., SUN, Y.-Z. et al., *J. Immunol.*, 1985, **135**, 3122.

EXPLORATIONS INTO THE ROLE OF SOMATIC MUTATION IN THE GENERATION OF ANTI-DNA ANTIBODIES

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Many studies over the past several years have shown that normal individuals can produce autoreactive antibodies [1-3]. These antibodies have been elicited *in vitro* by stimulation of B cells with polyclonal activators, and a large number of hybridoma lines making

autoreactive antibodies have been generated by fusion of normal human tonsillar or peripheral blood B cells or normal murine splenic B cells to continuously growing B-cell lines. These antibodies are, in general, of the IgM isotype, have low affinity for antigen,

and many seem to be polyspecific and bind to a variety of autoantigens [3, 4]. It has been suggested that these antibodies reflect germline gene sequences [5]. The high frequency with which hybridomas producing such autoantibodies can be generated suggests that they comprise a substantial proportion of the germline immunoglobulin gene repertoire. While it seems that all individuals can produce these antibodies when their B cells are activated with polyclonal B-cell activators, or when individual B cells are captured by hybridoma technology, only a very few individuals produce *in vivo* pathogenic autoantibodies causing autoimmune disease. The autoantibodies of autoimmune disease differ from other autoreactive antibodies in that they are almost exclusively IgG antibodies. Because an important feature of IgG antibodies is that they rarely reflect germline immunoglobulin gene sequences but almost always reflect the acquisition of somatic mutations [6], we have been studying whether the autoantibodies of autoimmune disease are generated by somatic mutation of germline gene sequences. We are exploring the molecular genetic origins of anti-double-stranded-DNA antibodies using two idiotypic systems: the 3I idiotypic system of human anti-DNA antibodies and the T15 system of murine anti-DNA antibodies.

3I reactive anti-DNA antibodies.

We have previously reported the generation of a monoclonal anti-idiotype, 3I, reactive with anti-double-stranded DNA antibodies isolated from a patient with systemic lupus erythematosus [7]. This anti-idiotype recognizes a determinant on κ light chains of anti-DNA antibodies in 85 % of patients with systemic lupus. In four patients studied, 3I reactive antibodies comprised from 40 to 90 % of the anti-DNA antibodies present in the serum [7]. In addition, 3I reactive antibodies are present in kidney biopsies of lupus patients. These data suggest that the 3I anti-idiotype recognizes a cross-reactive idiotype on a substantial percent of anti-DNA antibodies in a majority of

patients with lupus, and that these 3I reactive antibodies are pathogenic autoantibodies.

3I reactive antibodies that do not bind to double-stranded DNA are present in low titre in normal individuals and in high titre in relatives of patients with familial SLE [8]. Presumably, they form part of a normal non-autoimmune immune response and are reactive with microbial antigens. In order to determine the structural basis for DNA binding within the 3I idiotype system, we decided to analyse myeloma proteins for expression of the 3I idiotype. A total of 717 sera containing monoclonal immunoglobulin were studied; 82 demonstrated high titred 3I reactivity: 27 IgM myelomas, 47 IgG myelomas and 8 IgA myelomas. In each case, we could demonstrate that the 3I reactivity was a feature of the monoclonal protein and not of polyclonal immunoglobulins present in the sera. Of these 82 3I reactive monoclonal proteins, 29 could be shown to bind DNA using an assay in which sera are displayed on isoelectric focusing gels, transferred to nitrocellulose and probed with radiolabelled DNA. In this way, it was possible to show that the monoclonal protein itself was DNA-binding. When the DNA-binding proteins were analysed for heavy-chain isotype, we found 2 of 27 IgM bound DNA, 25 of 47 IgG immunoglobulins and 2 of 8 IgA immunoglobulins. The increased incidence of DNA binding among the IgG rather than the IgM immunoglobulins is statistically significant ($p > 0.001$). In order to compare the heterogeneity of the 3I reactive κ light chains associated with the IgM and the IgG proteins, we displayed 3 groups of proteins on 2-D gels. When twenty-four 3I reactive non-DNA binding IgM proteins were isolated on a 3I affinity column and displayed on 2-D gels, we found the κ light chains to be highly homogeneous. When 22 IgG non-DNA-binding immunoglobulins and 25 IgG DNA-binding myelomas were treated in a similar fashion, increased charge heterogeneity of the 3I reactive kappa light chains was apparent.

It seems likely to us that the IgM 3I reactive antibodies reflect germline

immunoglobulin gene sequences and show no reactivity with DNA. The IgG proteins may reflect the accumulation of somatic mutation, as evidenced by the increased charge heterogeneity of the κ light chains. It may be that, in this idiotype system, a high percent of mutations leads to the acquisition of affinity for double-stranded DNA. While several explanations of these data are possible, we believe the most likely one to be that, within the 3I idiotype system which includes pathogenic anti-DNA antibodies found in serum and tissue of patients with an autoimmune disease, the acquisition of autoreactivity is a consequence of somatic mutation of germline immunoglobulin genes. The unmutated IgM proteins show no specificity for DNA; the mutated IgG proteins show increased charge heterogeneity and a large number of them bind DNA. We hope that the amino acid sequences of the monoclonal proteins and the analysis of the genes encoding these antibodies will determine the validity of this hypothesis.

The T15 idiotype system.

We have previously shown, in collaboration with Dr M. Scharff, that a single amino acid substitution of the S107 anti-phosphorylcholine antibody can generate an antibody, U4, with reduced affinity for phosphorylcholine but with a newly acquired specificity for double-stranded DNA [9]. It is clear in this instance that the T15 germ line heavy and light chain immunoglobulin genes encode the S107 protein, an anti-bacterial antibody, and that somatic mutations lead to autoreactivity. To determine whether T15 positive anti-DNA antibodies are present in the serum of mice with lupus-like syndromes, we analysed anti-DNA antibodies from MRL/lpr and NZB/W mice for reactivity with an anti-T15 antibody. In serum for each of three mice tested from each strain, we found T15 anti-DNA antibodies present. When MRL/lpr mice are immunized with the bacterial antigen phosphorylcholine, their serum contains T15 anti-phosphorylcholine

antibodies and increased titres of T15 anti-DNA antibodies. This observation suggests that exposure to a bacterial antigen can lead to the production of autoantibodies bearing the idiotype of the antibacterial antibodies. We are currently studying whether the anti-DNA antibodies are cross-reactive with phosphorylcholine or whether they represent a separate population of antibodies that may be elicited through an idiotypic network.

Recently, we have developed a protocol for generating T15 anti-DNA antibodies in BALB/c mice, a non-auto-immune strain of mouse. BALB/c mice immunized with anti-I-J antibody produce high titres of T15 anti-DNA antibodies presumably because they no longer have adequate T suppressor cell activity. Of the hybridomas derived from these mice that make IgG antibodies with specificity for double-stranded DNA, many can be shown by RNA dot blot to use a T15 heavy-chain variable region gene. Sequencing of these hybridomas should reveal whether autoreactivity results from abnormal associations of heavy and light chains, from «forbidden» VDJ combinations or from somatic mutation. The example of the U4 antibody suggests that at least some may reflect somatic mutation.

Summary.

We feel that in these two idiotypic systems, one in humans and one in mice, there is data to suggest that pathogenic IgG autoantibodies may be generated by somatic mutation of germline immunoglobulin genes encoding non-autoreactive antibodies. These data may differ from data obtained in other systems because we have concentrated on the study of pathogenic IgG autoantibodies. It is our belief that the pathogenic autoantibodies of autoimmune disease differ from the germline-gene-encoded autoantibodies which have been described with respect to their heavy chain isotype and their affinity for antigen. We believe the autoantibodies of autoimmune disease are IgG antibodies and display high affinity for

antigen. The IgG antibodies may be produced by somatic mutation, and their expression in autoimmune individuals may reflect predominant use of a

particular germline gene or gene family as well as a defect in immunoregulation. Further studies are necessary to test these hypotheses.

References.

- [1] CAIRNS, E., BLOCK, J. & BELL, D.A., Anti-DNA autoantibody-producing hybridomas of normal human lymphoid cell origin. *J. clin. Invest.*, 1984, **74**, 880-887.
- [2] RAUCH, J., MASSOCOTTE, H. & TANNENBAUM, H., Specific and shared idiotypes found on hybridoma anti-DNA autoantibodies derived from rheumatoid arthritis and systemic lupus erythematosus patients. *J. Immunol.*, 1985, **133**, 489-494.
- [3] SATOH, J., PRABHAKAR, B.S., HASPEL, M.V., GINSBERG-FELLNER, F. & NOTKINS, A.L., Human monoclonal autoantibodies that react with multiple endocrine organs. *New Eng. J. Med.*, 1983, **309**, 217-220.
- [4] SHOENFELD, Y., ISENBERG, D.A., RAUCH, J., MADAIO, M.P., STOLLAR, B.D. & SCHWARTZ, R.S., Idiotypic cross-reactions of monoclonal human lupus autoantibodies. *J. exp. Med.*, 1983, **158**, 718-730.
- [5] NAPARSTEK, Y., DUGGAN, D., SCHATTNER, A., MADAIO, M.P., GONI, F., FRANGIONE, B., STOLLAR, B.D., KABAT, E.A. & SCHWARTZ, R.S., Immunochemical similarities between monoclonal antibacterial Waldenstrom's macroglobulins and monoclonal anti-DNA lupus autoantibodies. *J. exp. Med.*, 1985, **161**, 1525-1540.
- [6] GEARHART, P., JOHNSON, M.D., DOUGLAS, R. & HOOD, L., IgG antibodies to phosphorylcholine exhibit more diversity than their IgM counterparts. *Nature (Lond.)*, 1981, **291**, 28-34.
- [7] SOLOMON, G., SCHIFFENBAUER, J., KEISER, H.D. & DIAMOND, B., Use of monoclonal antibodies to identify shared idiotypes on human antibodies to native DNA from patients with systemic lupus erythematosus. *Proc. nat. Acad. Sci. (Wash.)*, 1983, **80**, 850-854.
- [8] HALPERN, R., DAVIDSON, A., LAZO, A., SOLOMON, G., LAHITA, R. & DIAMOND, B., Familial SLE. Presence of a cross-reactive idiomorph in healthy family members. *J. clin. Invest.* 1985, **76**, 731-736.
- [9] DIAMOND, B. & SCHARFF, M.D., Somatic mutation of the T15 heavy chain gives rise to an antibody with autoantibody specificity. *Proc. nat. Acad. Sci. (Wash.)* 1984, **81**, 5841-5844.

LYMPHOCYTE CHIMAERISM AND ANTI-DNA RESPONSE

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The spontaneous production of antibodies which react with DNA is classically associated with a particular genetic background in animal models, as in human diseases. A variety of hypotheses have been proposed to explain this phenomenon, including deficiencies of T-cell suppressor functions, excessive T-cell help or abnormal B-cell activity. In fact, anti-DNA antibodies might be primarily directed against other cross-reactive epitopes of bacterial origin, which may represent the effective triggering event for anti-DNA B-cell activation.

However, in several other animal models of lupus-like disease, anti-DNA antibodies were easily induced in various mouse strains which did not show any predisposition to autoimmunity. Thus, anti-DNA antibodies were found in mice undergoing intense polyclonal B-cell activation after injection of bacterial lipopolysaccharide (LPS) or after infection with *Escherichia coli*, *Trypanosoma brucei* or *Plasmodium falciparum*. In the first 24 h following injection of LPS, there is a release of DNA in circulating blood, and it is likely that combined activating signals from both DNA and LPS contribute to the generation of anti-DNA-producing cells. In these situations, the anti-DNA antibodies produced are mainly representative of T-independent isotypes with a predominance of IgG3 and IgM antibodies [7].

Information on other mechanisms involved in the triggering of anti-DNA antibodies in individuals with a «non-autoimmune» genetic background have also been provided by the analysis of *autoimmune syndromes associated with the co-existence of allogeneic lymphoid cell populations* in some patients or in

appropriate animal models. The immunological aggression characterizing graft-vs-host (GVH) disease is well known to trigger anti-DNA antibodies. This was particularly demonstrated in subacute GVH models by Gleichmann and his collaborators [3, 12], who pointed out the selective triggering of certain autoantibodies as compared to other classical markers of polyclonal B-cell activation. Anti-DNA antibodies appeared to result from the stimulation by parental alloreactive T cells of DNA-specific B cells originating from the F₁ host. The requirement and the nature of putative antigens involved in the induction of those antibodies have not yet been determined.

In the absence of any immunological aggression against host tissues, we have observed the occurrence of an autoimmune syndrome resembling GVH in BALB/c mice rendered tolerant to H2b alloantigens after neonatal injection of spleen cells from adult semi-allogeneic (BALB/c × C57BL/6) F₁ mice [4]. In this model, it was previously shown that some pathological manifestations, defined as *host-versus-graft disease*, can occur. Thus, splenomegaly was described in tolerant mice [13] and some tissue lesions were observed after repeated injections of F₁ cells [6]. In our experiments, we have seen that all mice in which tolerance was effectively induced developed an autoimmune syndrome characterized by antinuclear, anti-DNA and anti-thymocyte antibodies, by renal lesions and by IgG deposits in glomeruli, in choroid plexus and at the dermoepidermal junction.

The mechanisms responsible for the triggering of the anti-DNA antibodies after induction of neonatal tolerance were investigated [8].

Injection of semiallogeneic spleen cells into newborn mice led to *polyclonal B-cell activation*. Indeed, in the first weeks of life there was the appearance in tolerant mice of anti-hapten (DNP, FITC) antibodies associated with a marked hypergammaglobulinaemia. However, this polyclonal B-cell activation differed from that induced by LPS: the predominant IgG subclass observed in tolerant mice was IgG1. However, polyclonal B-cell activation could hardly account for the full development of the associated autoantibodies. Although anti-DNA antibodies appeared with a kinetic pattern very similar to that of anti-DNP or anti-FITC antibodies, their level was much higher than what could be expected in a situation of polyclonal B-cell activation: the titre of IgG1 anti-DNA was in the range of 1/400 to 1/800 in mice injected with LPS, but reached 1/10,000 to 1/20,000 after induction of neonatal tolerance. On the contrary, anti-DNP or anti-FITC titres were similar in both situations. Therefore, the possibility should be considered that the development of anti-DNA antibodies after induction of neonatal tolerance may reflect the combination of an antigen-specific B-cell triggering with a polyclonal B-cell activation. In that case, the specific antigenic stimulation may result either from a DNA release associated with cell death during the early phase of tolerance induction, or from a priming by endogenous bacterial phospholipids cross-reacting with DNA.

Second, the *correlation between induction of autoimmunity and the possible establishment of B-cell chimaerism* was studied.

Immunoglobulins from BALB/c mice bear the Ig^a allotype, while those of C57BL/6 mice bear the Ig^b allotype. The presence in serum of immunoglobulins bearing the b allotype, indicating the persistence of donor F₁ Ig-producing cells, was demonstrated in all tolerant mice. When different numbers of donor cells were used for neonatal injection, the development of anti-DNA antibodies was shown to be closely linked with the establishment of tolerance, as evaluated by CTL-P

frequency and with the persistence of a sufficient number of B cells from the F₁ donor. Indeed, anti-DNA antibodies were seen only in those mice which exhibited a detectable level of Ig^b allotype.

Advantage was also taken of the existing allotypic differences between F₁ donor and newborn recipient mice to identify the *origin of the autoantibody-producing cells*. Experiments were performed in which the F₁ mice used resulted from a crossing between C57BL/6 mice and congenic BALB/c mice with the Ig^b allotype (BALB. Ig^b). Such F₁ mice produce immunoglobulins bearing only the Ig^b allotype. After transfer of spleen cells from these mice to conventional BALB/Ig^a newborn mice, there was a persistence of relatively high levels of Ig^b-bearing immunoglobulins, up to 20 weeks, while the level of Ig^a-bearing immunoglobulins was not significantly changed as compared to BALB/c control mice. Anti-DNA antibodies bearing the Ig^b allotype were detected at high levels, while there were no detectable Ig^a bearing anti-DNA antibodies. Therefore, in this situation, all anti-DNA antibodies appeared to be produced by F₁ donor cells. It should be noted that the anti-DNP and anti-FITC antibodies which were detected in this experiment also appeared to be mostly of the Ig^b allotype.

The mechanisms responsible for the *activation of donor B cells* in the newborn recipient have been partly elucidated. There is evidence that it is a T-cell-dependent phenomenon, as was first suggested by the predominance of IgG1 in anti-hapten and anti-DNA responses. There were no detectable anti-DNA antibodies in athymic nu/nu BALB/c mice which received a neonatal injection of semi-allogeneic F₁ cells, although there was evidence of persisting F₁ B cells. Furthermore, the same mice were shown to develop high levels of IgG1 anti-DNA antibodies (Ig^b allotype) after one injection of L3T4+T cells from nu/+ BALB/c mice, at 3 weeks of age. In addition, treatment of other tolerant BALB/c mice with anti-L3T4 monoclonal antibodies inhibited the generation of anti-DNA antibodies [9].

It is known that, whereas only a small number of IL-2 producing T cells able to recognize allo-MHC determinants exist in the spleen of newborn mice [10], a relatively high frequency of cells reacting to allogeneic stimulation can be found in lymph nodes of 6-day old mice. This may initially be sufficient, in our model, to activate and help the semi-allogeneic B cells in the weeks following the induction of tolerance. In fact, the tolerant state was found to be associated with a 10-fold reduction of the number of IL-2-producing T-cell precursors, but not to a complete disappearance of these cells [2, 14]. Furthermore, many investigators have implicated suppressor T cells in the maintenance of neonatal tolerance [5]. Along this line, it is conceivable that occasional disturbances of such regulatory mechanisms may lead to the exposure of persistent semi-allogeneic donor B cells to alloreactive host T cells.

Can autoimmunity result from an unsuspected chimaerism?

Similarities exist between the appearance of autoreactive donor B cells in

tolerant mice and the induction of a lupus-like disease in chronic models of GVH disease. In both situations, IgG autoantibodies are observed and there is a preferential stimulation of anti-DNA antibodies as compared to anti-hapten antibodies. The main difference between these models is the existence of GVH of interactions between the transferred T cells and all semi-allogeneic host cells whereas, in the neonatal tolerance model, allogeneic effects are limited to the chimaeric lymphoid cell populations.

The observation of autoimmune features associated with the establishment of lymphoid chimaerism in tolerant mice is of relevance for the understanding of the pathological manifestations appearing in chimaeric children [1]. In addition, a maternofetal transplacental passage of lymphocytes has been shown to occur occasionally at the time of delivery and this may represent a source of lymphocyte chimaerism in man with a potential delayed pathogenic effect [11]. It is conceivable that such «physiological» chimaerism may remain completely unsuspected and be responsible for autoimmune manifestations in some individuals.

References.

- [1] CIVIDALLI, G., YATZIV, S., VOSS, R., GALILI, V., KOPOLOVIC, J., SAGI, M., WEIZMAN, Z., BRAUTBAR, C. & KOHN, G., XX/XY lymphoid chimerism in a boy with fatal lymphohistiocytic proliferation. *J. Amer. med. Ass.* 1981, **246**, 1917.
- [2] FENG, H.M., GLASEBROOK, A.L., ENGERS, H.D. & LOUIS, J.A., Clonal analysis of T-cell unresponsiveness to alloantigens induced by neonatal injection of F₁ spleen cells into parental mice. *J. Immunol.*, 1983, **131**, 2165.
- [3] GLEICHMANN, E., GLEICHMANN, H. & WILKE, W., Autoimmunization and lymphomagenesis in parent-F₁ combinations differing at the major histocompatibility complex: model for spontaneous disease caused by altered self-antigens? *Transplant. Rev.*, 1976, **31**, 156.
- [4] GOLDMAN, M., FENG, H., ENGERS, H., HOCHMANN, A., LOUIS, J. & LAMBERT, P.H., Autoimmunity and immune complex disease after neonatal induction of transplantation tolerance in mice. *J. Immunol.*, 1983, **131**, 251.
- [5] GORCZYNSKI, R.M. & MACRAE, S., Suppression of cytotoxic response to histocompatible cells.-II. Analysis of the role of two independent T suppressor pools in maintenance of neonatally induced allograft tolerance in mice. *J. Immunol.*, 1979, **122**, 747.
- [6] HARD, R.C. & KULLGREN, B., Etiology, pathogenesis of a fatal graft-versus-host syndrome in parent/F₁ mouse chimeras. *Amer. J. Path.*, 1970, **59**, 203.

- [7] IZUI, S.A., EISENBERG, R.A. & DIXON, F., Subclass restricted IgG polyclonal antibody production in mice injected with lipid-A-rich lipopolysaccharides. *J. exp. Med.*, 1981, **153**, 324.
- [8] LUZUY, S., MERINO, J., ENGERS, H., IZUI, S. & LAMBERT, P.H., Autoimmunity after induction of neonatal tolerance to alloantigens: role of B-cell chimerism and F₁ donor B-cell activation. *J. Immunol.*, 1986, (in press).
- [9] MERINO, J., LUZUY, S., DUCHOSAL, M.A., PIGUET, P.F., IZUI, S. & LAMBERT, P.H., Autoimmunity after induction of neonatal tolerance to alloantigens. – II. role of recipient T cells in the activation of auto-reactive B cells (submitted).
- [10] PIGUET, P.F., IRLE, C., KOLLATTE, E. & VASSALLI, P., Post-thymic T-lymphocyte maturation during ontogenesis. *J. exp. Med.*, 1981, **154**, 581.
- [11] RAJENDRA, G.D. & CREGER, W.P., Maternofetal passage of leukocytes and platelets in man. *J. Hematology*, 1963, **21**, 6.
- [12] ROLINK, A.G., RADASZKIEWICZ, T., PABS, S.T., VAN DEN MEER, W.G.J. & GLEICHMANN, E., 1982. Allosuppressor and allohelper T cells in acute and chronic graft-versus-host disease. – I. Alloreactive suppressor cells rather than killer T cells appear to be the decisive effector cells in lethal graft-versus-host disease. *J. exp. Med.*, 1982, **155**, 1501.
- [13] SIMPSON, E., HOPP, O., HARRISON, S., MOSIER, M., MELIEF, D. & CANTOR, H., Immunological disease induced by injecting F₁ lymphoid cells into certain parental strains. *Immunology*, 1974, **27**, 289.
- [14] WATERFIELD, J.D., BICK, P.H., MÖLLER, E. & MÖLLER, G., Presence of alloantigen-reactive cells in neonatally tolerized mice. *Scand. J. Immunol.*, 1978, **7**, 435.

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THE ORIGIN OF ANTI-DNA ANTIBODY FORMATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Anti-DNA autoantibodies represent the most specific immunological marker of SLE. High titres of anti-double-stranded DNA are found nearly exclusively in the serum of SLE patients. This specificity to SLE, added to some level of correlation of antibody titre with disease activity and with the presence of the antibodies in the renal eluates of SLE patients and mice, have supported the hypothesis of the pathogenic role of these antibodies in SLE. The recent report by B. Hahr of a (transient) clinical improvement in B/W mice by anti-

DNA idotype manipulation [1] is in keeping with this hypothesis. However, seroclinical correlations are relative, most notably in severe cases without DNA binding activity or mild cases with high antibody titres resistant to immunosuppressive therapy. Furthermore, the presence of DNA within circulating immune complexes or renal eluates has always been elusive.

The problem has recently been complicated by the observation of a number of cross-reactivities between anti-DNA monoclonal antibodies and

various antigens. Thus, Schwartz *et al.* has reported that anti-DNA monoclonals of the IgM class, usually directed against single-stranded DNA, cross-react with phospholipids including the well known cardiolipin, responsible for the false-positive reactions with syphilis antigens often observed in SLE [2]. Our own group has reported the cross-reaction of anti-ds DNA monoclonals with a protein present on the membrane of a number of mammalian cells involved in lupus pathogenesis, including normal human glomeruli, T and B lymphoblastoid cell lines, erythrocytes, platelets and neuronal tissue [3, 4]. This protein has now been characterized by immunoblotting and immunoprecipitation analysis. Antigenic activity resides in several polypeptides with respective MW of 14, 16, 17, 33 and Kd [5]. The question must be raised as to the nature of these cross-reactivities. They could be due to a chance sharing of a precise epitope, as has been described between unrelated monoclonals [6]. Alternatively, and much more likely in our case, the cross-reaction is explained by a similarity in the conformation of dsDNA and the protein. This interpretation is suggested by the consistency of the cross-reaction found with all anti-dsDNA monoclonal antibodies tested. The reason for this conformational analogy remains to be determined.

These observations pose two basic questions: 1) is DNA the triggering antigen in SLE? 2) is DNA the meaningful target of «anti-DNA antibodies» in SLE pathogenesis?

It is well established that high-level immune responses require the persistent presence of the specific antigen. This notion probably applies to autoimmune responses, since neonatal thyroidectomy

prevents the anti-thyroglobulin autoantibody response normally observed in the spontaneously autoimmune obese strain of chicken. If this is the case for anti-DNA antibodies in SLE, is DNA the triggering antigen? DNA is indeed released by dying cells and is a constituent of a number of viruses. The difficulties met in inducing anti-DNA antibody production by deliberate immunization with nucleic acids does not favour this hypothesis. Alternatively, one may hypothesize that phospholipids represent the triggering mechanism. In the case of each hypothesis, however, one must explain why SLE patients produce a whole spectrum of antibodies directed against nuclear antigens (distinct from dsDNA). It is possible that other conformational analogues similar to that described above exist.

The problem of the target antigen is posed in a similar fashion. Anti-DNA (anti-protein) antibodies could combine with DNA (even if the production were initially elicited by the protein) and form pathogenic immune complexes. The fact that the presence of DNA within SLE immune complexes or renal eluates has not been conclusively and consistently demonstrated does not argue in favour of this hypothesis. Alternatively, the antibodies could bind to the cells, expressing the protein, or could contribute to forming circulating immune complexes with the protein. In any case, the two hypotheses are not mutually exclusive.

In conclusion, the questions of the origin and of the pathogenic role of anti-DNA antibodies on SLE are posed in new terms. One can assume that another antigen (phospholipids or cell-surface protein(s)) could be the triggering antigen rather than DNA itself.

References.

- [1] HAHN, B.H. & EBLING, F.M., Suppression of NZB X NZW murine nephritis by administration of a syngeneic monoclonal anti-DNA to DNA. Possible role of anti-idiotypic antibodies. *J. Clin. Invest.* 1983, **71**, 1728.
- [2] LAFER, E.M., RAUCH, J., ANDRZEJEWSKI, C., MUDD, D., FURIE, B., SCHWARTZ, R.S. & SOLLAR, B.D., Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J. exp. Med.*, 1981, **153**, 897.

- [3] IRON, F., JACOB, L. & BACH, J.F., A. monoclonal anti-DNA autoantibody binds to Raji cells: implications for the interpretation of the Raji cell assay for immune complexes. *Europ. J. Immunol.*, 1984, **14**, 283.
- [4] JACOB, L., LETY, M.A., LOUVARD, D. & BACH, J.F., Binding of a monoclonal anti-DNA autoantibody to identical protein(s) present at the surface of several human cell types involved in lupus pathogenesis. *J. clin. Invest.*, 1985, **75**, 315.
- [5] JACOB, L., IRON, F., BACH, J.F. & LOUVARD, D., A monoclonal anti-DNA autoantibody also binds to cell-surface protein(s). *Proc. nat. Acad. Sci. (Wash.)*, 1984, **81**, 3843.
- [6] SATOH, J., PRABHAKAR, B.S., HASPEL, M.V., GINSBERG-FELLNER, F. & NOTKINS, A.L., Human monoclonal autoantibodies that react with multiple endocrine organs. *New Engl. J. Med.*, 1983, **309**, 217-220.

**AUTOANTIGEN PRESENTATION BY TARGET CELLS:
ITS POSSIBLE ROLE IN DETERMINING
AUTOANTIBODY SPECIFICITY**

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The autoimmune diseases form a broad spectrum of disorders ranging from those conditions in which the attack is clearly organ-specific (*e.g.* most autoimmune endocrine diseases, including autoimmune thyroid diseases or ATD) [5] to those which are relatively non-organ-specific (*e.g.* systemic lupus erythematosus or SLE) [26]. This classification also applies to the autoantibodies found in patients with these diseases, with antibodies reacting solely to thyroid constituents being present in the former example, whereas antibodies to ubiquitous cell components like nucleic acids and associated proteins characterize the latter. One of the prime factors governing the position of an individual patient within the above spectrum is almost certainly the nature of the autoantigen(s) involved and the means whereby they are presented to autoreactive T lymphocytes.

HLA class II⁺ epithelium and the targeting of the response.

The immunogenic presentation of antigens to helper T cells is normally achieved in the context of major histocompatibility complex (MHC) class II molecules. Most cells of the body other than those normally involved in immune activities do not usually express class II products. For example, thyroid epithelial cells (thyrocytes) are normally class II⁻; they do, however, have the capacity to express class II products when stimulated *in vitro* with lectins [22] or interferon(IFN)-gamma [32]. Most importantly, these cells synthesize class II molecules in the majority of patients with thyroid autoimmunity [12]. Such expression might enable thyrocytes to function as antigen-presenting cells [6]: indeed, *in vitro* experiments have demonstrated the

functional ability of class II⁺ thyrocytes to effectively present exogenous antigenic peptides [19] and also stimulate cloned autoreactive T-cell lines derived from the infiltrate of autoimmune thyroid. This was achieved in an antigen-specific, MHC class II-restricted fashion [18]. It therefore appears that class II expression by thyrocytes enables them to present their own surface molecules as autoantigens, thus bypassing requirements for «conventional» antigen-presenting cells [9]. A need for additional accessory signals in autostimulation, such as those provided by interleukin-1 (IL-1), is presently unknown. However, given the variety of cell types, including epithelial cells, known to produce IL-1-like factors [21], it appears probable that thyrocytes could also fulfill these requirements.

Autoantigen presentation by class II⁺ thyrocytes is very probably important in potentiating and propagating the autoimmune attack. Its role in the initiation of autoimmunity is presently unclear, although the involvement of non-inherited factors is suggested by incomplete concordance in identical twins with autoimmunity [3]. However, the wide applicability of this model is suggested by the growing list of autoimmune diseases in which organ-specific epithelial class II expression has been described (reviewed in [33]). A similar model for autoantigen presentation by class II⁺ thyrocytes has been developed in mice [7].

Any model of organ-specific autoimmunity must take account of the diversity of the autoantibodies produced at the same time as explaining the restriction of reactivity against a single organ or even a cell-type within that organ [29]. For example, an individual with thyroid autoimmunity can possess autoantibodies to the thyroid-stimulating hormone receptor (TSH-R), thyroid microsomal antigen (TM) and thyroglobulin (Tg). Since these three molecules do not appear to share biochemical or immunological properties, and yet antibodies to other autoantigens present in the gland occur much less frequently in ATD, an imperative question

is what feature makes only these molecules immunogenic [23]? One property which is clearly common to all three is that they are surface membrane molecules of the thyroid epithelial cells [27, 15, 10], including Tg in its precursor form [24]. Furthermore, they are present in all normal individuals.

The nature of the antibody response in thyroid autoimmunity, *i.e.* to TSH-R, TM and Tg, can thus be explained by *in situ* presentation of these molecules, apparently in their native form, by thyrocytes co-expressing class II molecules. Support for this concept is provided by our recent findings of significant associations between the occurrence in individual patients of class II⁺ thyrocytes and serum thyroid autoantibodies. More specifically, the presence of anti-TM is particularly associated with HLA-DR expression by thyrocytes and anti-Tg with HLA-DQ [31].

With regard to the B cells producing autoantibodies, McLachlan *et al.* [20] have found that intrathyroidal lymphocytes spontaneously producing autoantibodies upon isolation appear to be intimately associated with the thyroid follicles. This again supports the concept of autoimmune activation occurring at the thyrocyte surface.

Possible relationships between different mechanisms of autoantibody production.

A further point to be borne in mind is that thyroid autoimmunity itself has a variety of clinical manifestations with differential occurrence of autoantibodies. Thus, anti-TSH-R are characteristic of Graves' disease, where their ability to mimic the action of TSH leads to hyperthyroidism. By contrast, anti-TM and anti-Tg are most commonly associated with autoimmune thyroiditis, which leads to hypothyroidism in destructive Hashimoto's disease. Furthermore, Graves' disease is associated with HLA-DR3, whereas Hashimoto's disease has recently been recognized to be associated with HLA-DR4 (N. Farid, personal communication) rather than with HLA-DR5, as previously suggested [8]. Different conditions may the-

refore be involved in the generation of anti-TSH-R on the one hand, and anti-TM and anti-Tg on the other. One possibility is that TSH-R is most efficiently presented by thyrocytes expressing HLA-DR3-associated gene products, while HLA-DR4 favours the presentation of TM and Tg. However, TSH-R constitutes something of a «special case» amongst thyroid autoantigens, since it is a hormone receptor.

Alternative mechanisms to that proposed above for the generation of anti-TSH-R may therefore be relevant. For example, anti-TSH-R from Graves' patients have been found to cross-react with the Gram-negative enteric bacterium *Yersinia enterocolitica*, raising the possibility that bacterial infection could generate anti-TSH-R via «molecular mimicry» [34, 13]. Another possibility is raised by the finding that rabbits or mice immunized with TSH not only develop anti-TSH antibodies but also thyroid-stimulating antibodies against TSH [4, 11]. Thus, by one means or another, anti-TSH-R could be generated by a mechanism not necessarily requiring class II expression by thyrocytes. However, since anti-TSH-R can mimic the activity of TSH, it is relevant to note our recent finding that treatment with TSH can greatly augment class II expression by normal thyrocytes stimulated with IFN-gamma [30]. Is it possible that stimulating TSH-R antibodies have similar effects? This is presently under investigation and has also been demonstrated by other workers (B.E. Wenzel, personal communication). Thus, one could envisage that anti-TSH-R antibodies in Graves' disease may facilitate thyrocyte class II expression, which would lead to an antibody response to TM and Tg and ultimately to autoimmune thyroiditis. This could explain the broad overlap between Graves' disease and autoimmune thyroiditis, and the observation that the former disease can develop into the latter. However, whichever (if any) of the above mechanisms for the generation of anti-TSH-R is correct, an important feature is the concentration of events at the thyrocyte surface, with thyrocyte class II expression being a key pathogenic feature.

The role of class II expression across the spectrum of autoimmunity.

The model we have proposed for the role of class II⁺ epithelial cells in organ-specific autoimmunity bears both similarities to and differences from models relating to those autoimmune diseases characterized by non-organ-specific autoantibodies. For example, Rosenberg *et al.* [25] proposed that an important feature of the autoimmune syndrome in MRL mice is the raised numbers of Ia⁺ macrophages and B cells which, together with T cells spontaneously producing IFN-gamma, set up a self-perpetuating autologous mixed-lymphocyte reaction. Also, in joints affected by rheumatoid arthritis, the large numbers of class II⁺ macrophages/dendritic cells are thought to stimulate a similar circuit of T-cell activation [14, 16].

A clear difference between the models relating to organ-specific and non-organ-specific autoimmunity is the nature of the class II⁺-presenting cell, *i.e.* organ-specific epithelial cells *versus* «conventional» specialized immunocytes. To return to our opening comments, this difference could well determine the nature of the autoantigen(s) against which antibodies are made. This is exemplified by the possibility of presentation by thyroid epithelium being instrumental in the generation of anti-TM and anti-Tg, but macrophages and/or dendritic cells being important for anti-DNA or rheumatoid factor production. However, many autoimmune diseases cannot be strictly defined as being either organ-specific or non-organ-specific, in which case both presentation mechanisms may be operative to varying degrees. For example, in primary biliary cirrhosis and primary Sjogren's syndrome, in which non-organ-specific autoantibodies are often produced, the observed expression of class II molecules by bile duct [2] and salivary gland epithelium [17], respectively, may contribute to the localization of damage to these tissues. A variety of factors could determine whether the balance is shifted towards epithelial or «conventional» antigen presentation. This is supported by the

observations that class II expression by thyrocytes is enhanced by substances stimulating cyclic AMP production and suppressed by epidermal growth factor, whereas these factors have the converse effects on class II expression by macrophages [28, 1].

However, a clear similarity between all the models discussed above is that they involve inappropriate class II expression: be it aberrant expression by endocrine epithelial cells or excessive numbers of class II⁺ macrophage/dendritic cells.

References.

- [1] ACRES, R.B., LAMB, J.R. & FELDMANN, M., Effects of platelet-derived growth factor and epidermal growth factor on antigen-induced proliferation of human T-cell lines. *Immunology*, 1985, **54**, 9-16.
- [2] BALLARDINI, G., MIRAKIAN, R., BIANCHI, F.B., PISI, E., DONIACH, D. & BOTTAZZO, G.F., Aberrant expression of HLA-DR antigens on bile duct epithelium in primary biliary cirrhosis: relevance to pathogenesis. *Lancet*, 1984, **II**, 1009-1013.
- [3] BARNETT, A.H. *et al.*, Diabetes in identical twins: a study of 200 pairs. *Diabetologia*, 1981, **20**, 87-93.
- [4] BEALL, G.N., RAPOPORT, B., CHOPRA, I.J. & KRUGER, S.R., Further studies of the production by rabbits of thyroid-stimulating antiidiotypic antibodies to TSH, in «Proceedings of the 9th International Thyroid Congress, Sao Paulo, Sept. 1985». Plenum Press, New York, 1986 (in press).
- [5] BOTTAZZO, G.F. & DONIACH, D., Polyendocrine autoimmunity: an extended concept, in «Autoimmunity and endocrine disease» (R. Volpe) (pp. 375-403). Marcel Dekker, New York, 1985.
- [6] BOTTAZZO, G.F., PUJOL-BORRELL, R., HANAFUSA, T. & FELDMANN, M., Role of aberrant HLA-DR expression and antigen presentation in the induction of endocrine autoimmunity. *Lancet*, 1983, **II**, 1115-1119.
- [7] CHARREIRE, J. & SALAMERO, J., Possible target antigens in autoimmune endocrine disease. *Immunol. Today*, 1984, **5**, 337.
- [8] FARID, N.R., SAMPSON, L., MOENS, H. & BARNARD, J.M., The association of goitrous autoimmune thyroiditis with HLA-DR5. *Tissue Antigens*, 1981, **17**, 265-272.
- [9] FELDMANN, M., DONIACH, D. & BOTTAZZO, G.F., The Heterogeneity of autoimmune responses, in «Immunology of rheumatic diseases» (S. Gupta & N. Talal) (pp. 271-300). Plenum Press, New York, 1985.
- [10] FENZI, G.F., BARTALENA, L., CHIOVATO, L., MARCOCCI, C., ROTELLA, C.M., ZONEFRATI, R., TOCCAFONDI, R. & PINCHERA, A., Studies on thyroid cell surface antigens using cultured human thyroid cells. *Clin. exp. Immunol.*, 1982, **47**, 336-344.
- [11] GANFI, M., BEN DAVID, H., GORDON, A. & GROSS, J., The induction of immunoglobulins that stimulate thyroid growth, iodide concentration and cause TSH binding in the mouse, in «Proceedings of the 9th International Thyroid Congress, Sao Paulo, Sept. 1985». Plenum Press, New York, 1986 (in press).
- [12] HANAFUSA, T., PUJOL-BORRELL, R., CHIOVATO, L., RUSSELL, R.C.G., DONIACH, D. & BOTTAZZO, G.F., Aberrant expression of HLA-DR antigen on thyrocytes in Graves' disease: relevance for autoimmunity. *Lancet*, 1983, **II**, 1111-1115.
- [13] HEYMA, P. & HARRISON, L.C., Thyrotropin (TSH) binding sites on *Yersinia enterocolitica* recognized by immunoglobulins from humans with Graves' disease. *Clin. exp. Immunol.*, 1986 (in press).
- [14] JANOSSY, G., PANAYI, G., DUKE, O., BOFILL, M., PULTER, L.W. & GOLDSTEIN, G., Hypothesis: rheumatoid arthritis: a disease of T-lymphocyte/macrophage immunoregulation. *Lancet*, 1981, **II**, 839-842.

- [15] KHOURY, E.L., HAMMOND, L., BOTTAZZO, G.F. & DONIACH, D., Presence of the organ-specific «microsomal» autoantigen on the surface of human thyroid cells in culture: its involvement in complement-mediated cytotoxicity. *Clin. exp. Immunol.*, 1981, **45**, 316-328.
- [16] KLARESKOG, L., FORSUM, U., SCHEYNIUS, A., KABELITZ, D. & WIGZELL, H., Evidence in support of a self-perpetuating HLA-DR-dependent delayed-type cell reaction in rheumatoid arthritis. *Proc. nat. Acad. Sci. (Wash.)*, 1982, **79**, 3632-3636.
- [17] LINDAHL, G., HEDFORS, E., KLARESKOG, L. & FORSUM, U., Epithelial HLA-DR expression and T-lymphocyte subsets in salivary glands in Sjogren syndrome. *Clin. exp. Immunol.*, 1985, **61**, 475-482.
- [18] LONDEI, M., BOTTAZZO, G.F. & FELDMANN, M., Human T-cell clones from autoimmune thyroid gland: specific recognition of autologous thyroid cells. *Science*, 1985, **228**, 85-89.
- [19] LONDEI, M., LAMB, J.R., BOTTAZZO, G.F. & FELDMANN, M., Epithelial cells expressing aberrant MHC class II determinants can present antigen to cloned human T cells. *Nature (Lond.)*, 1984, **312**, 639-641.
- [20] MCLACHLAN, S.M., PEGG, C.A.S., ATHERTON, M.C., MIDDLETON, S.E., DICKINSON, A., CLARK, F., PROCTOR, S.J., PROUD, G. & REES SMITH, B., Subpopulations of thyroid autoantibody-secreting lymphocytes in Graves' and Hashimoto thyroid glands. *Clin. exp. Immunol.*, 1986 (in press).
- [21] OPPENHEIM, J.J., KOVACS, E.J., MATSUSHIMA, K. & DURUM, S.K., There is more than one interleukin 1. *Immunol. Today*, 1986, **7**, 45-56.
- [22] PUJOL-BORRELL, R., HANAFUSA, T., CHIOVATO, L. & BOTTAZZO, G.F., Lectin-induced expression of DR antigen on human cultured follicular thyroid cells. *Nature (Lond.)*, 1983, **303**, 71-73.
- [23] ROITT, I.M., Prevailing theories in autoimmune disorders. *Triangle*, 1984, **23**, 67-76.
- [24] ROITT, I.M., PUJOL-BORRELL, R., HANAFUSA, T., DELVES, P.J., BOTTAZZO, G.F. & KOHN, L.D., Asialoagalactothyroglobulin binds to the surface of human thyroid cells at a site distinct from the «microsomal» autoantigen. *Clin. exp. Immunol.*, 1984, **56**, 129-134.
- [25] ROSENBERG, Y.J., STEINBERG, A.D. & SANTORO, T.J., The basis of autoimmunity in MRL-lpr/lpr mice: a role for self Ia-reactive T cells. *Immunol. Today*, 1984, **5**, 64-67.
- [26] SCHERBAUM, W.A., BLASCHEK, M., BERG, P.A., DONIACH, D. & BOTTAZZO, G.F., Spectrum and profiles of non-organ-specific autoantibodies in autoimmune diseases, in «Immunocytochemistry: modern methods and applications» (J.M. Polack & S. Van Norden). John Wright — PSG Inc., Bristol, 1986 (in press).
- [27] SMITH, B.R. & HALL, R., Thyroid-stimulating immunoglobulins in Graves' disease. *Lancet*, 1974, **II**, 427-431.
- [28] SNYDER, D.S., BELLER, S.I. & UNANUE, E.R., Prostaglandins modulate macrophage Ia expression. *Nature (Lond.)*, 1982, **299**, 163-165.
- [29] TODD, I. & BOTTAZZO, G.F., Laboratory investigation of autoimmune endocrine diseases. *Clin. Immunol. Allergy*, 1985, **5**, 613-638.
- [30] TODD, I., McNALLY, J.M., HAMMOND, L.J. & PUJOL-BORRELL, R., TSH enhances expression by thyrocytes of interferon-gamma-induced HLA-D/DR, in «Proceedings of the 9th International Thyroid Congress, Sao Paulo, Sept. 1985». Plenum Press, New York, 1986 (in press).
- [31] TODD, I., LUCAS MARTIN, A., ABDUL-KARIM, B.A.S., HAMMOND, L.J. & BOTTAZZO, G.F., HLA-D subregion expression by thyrocytes is associated with the occurrence of circulating thyroid autoantibodies. *Ann. Endocrinol. (Abstract)*, 1986 (in press).
- [32] TODD, I., PUJOL-BORRELL, R., HAMMOND, L.J., BOTTAZZO, G.F. & FELDMANN, M., Interferon-gamma induces HLA-DR expression by thyroid epithelium. *Clin. exp. Immunol.*, 1985, **61**, 265-273.

- [33] TODD, I., PUJOL-BORRELL, R., LONDEI, M., FELDMANN, M. & BOTTAZZO, G.F., New ideas on the pathogenesis of autoimmune disease, in «Advanced Medicine 22» (D.R. Triger). Transmedica Europe Ltd, Tunbridge Wells, 1986 (in press).
- [34] WEISS, M., INGBAR, S.H., WINBLAD, S. & KASPER, D.L., Demonstration of a saturable binding site for thyrotropin in *Yersinia enterocolitica*. *Science*, 1983, **219**, 1331-1333.

LY-1 B CELLS AND AUTOANTIBODIES

LAH 286

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Two lineages of B lymphocytes can be distinguished in reconstitution experiments in lethally irradiated mice. One lineage, which includes most B cells in the spleen, lymph nodes and peripheral blood, is derived from progenitors found in the spleen and bone marrow throughout the life of the animal. The other derives from progenitors consistently present in adult peritoneum rather than in bone marrow. This latter (Ly-1 B) lineage is predominantly found in peritoneum and spleen [1].

Multiparameter analysis with the fluorescence-activated cell sorter (FACS) identifies cells of the Ly-1 B lineage as displaying, in addition to the Ly-1 antigen, a characteristic phenotype with respect to a number of classical B lymphocyte cell surface antigens. These cells express high IgM, low IgD, intermediate Ia and low B220 (as measured by the monoclonal antibody 6B2) [1] and A.M.S., (unpublished results). Ly-1 B cells represent only 1-2 % of splenic lymphocytes and are undetectable in lymph node, Peyer's patches and peripheral blood. In contrast, up to 70 % of peritoneal B cells (20-40 % of total lymphocytes) in normal mice are of the Ly-1 B lineage [2]. Ly-1 B cells occur early in ontogeny and can constitute the majority of B lymphocytes found in foetal liver and neonatal spleen [1] (see table I).

The conclusion that progenitors of Ly-1 B cells in the adult animal are distinct from progenitors of typical splenic and lymph node B cells rests on data from reconstitution experiments in which cells from various sources were transferred into lethally irradiated allotype (Igh) congenic recipients. One to six months later, the reconstituted B-cell populations were characterized by FACS and functional analyses [6]. These studies showed that IgM peritoneal B cells reconstitute only the Ly-1 B population in irradiated recipients. In contrast, progenitors found in adult bone marrow, which reconstitute virtually the entire B-cell population, contribute little or nothing to the repopulation of Ly-1 B cells. These data indicate that Ly-1 B cells are produced early in ontogeny and maintained as an immunoglobulin self-renewing population throughout the life of an individual.

In the spirit of this Forum, we would like to present the following observations and speculations regarding the relationship of Ly-1 B cells and autoantibodies.

- 1) **Ly-1 B cells produce high levels of «natural» serum antibody.**

FACS-purified peritoneal Ly-1 B cells constitutively produce IgM both *in*

TABLE I. — Ly-1 B cell characteristics.

Phenotype:	High IgM, Low IgD, Low B220 plus Ly-1. Increased frequency of lambda light chain expression.
Location:	Peritoneum >> spleen >> nodes, bone marrow (undetectable).
Ontogeny:	Highly enriched in neonates.
Tumours:	NFS-1 and NFS-5 [3], 7OZ (most isolates), BCL-1 [4] some Abelson pre-B tumour lines and the CH tumour series [5] have been identified as Ly-1 B cells.
Genetics:	Exclusive Motheaten Elevated NZB-related autoimmune mice High BALB-related mice (in peritoneum) Medium C57BL/10, CBA, C3H Low SJL-related Missing CBA/N, DBA/2Ha

vitro and when adoptively transferred into irradiated recipients ([7] and A.M.S., unpublished results). As few as 5×10^6 IgM⁺, Ly-1⁺ peritoneal cells produce normal serum levels of IgM and IgG2a within three weeks following transfer (AMS, unpublished results). At present, we cannot determine the relative contribution that Ly-1 B cells make to total serum Ig in an intact animal; however, it is clear that they are capable of producing most of this naturally occurring serum Ig.

2) Ly-1 B cells produce autoreactive «high connectivity» antibodies.

Hayakawa *et al.* have shown that, in NZB mice, autoantibodies to single-stranded DNA and to thymocyte surface antigens are exclusively produced by Ly-1 B cells. In normal mice, Ly-1 B cells are responsible for the production of autoantibodies recognizing bromelain-treated mouse red blood cells (BrmRBC) [7]. In addition, Leu-1 B cells, the human equivalent of Ly-1 B cells, have been associated with the production of cold haemagglutinins, rheumatoid factors and anti-cytoskeletal antibodies [8].

Recently, several laboratories have studied libraries of hybridomas produced from B cells obtained from foetal liver, neonatal spleen or peritoneum ([9,

10] and C. Bona, personal communication). Unlike hybridomas derived from adult spleen cells, a large number of these hybridomas produce antibodies which are highly autoreactive. Kearney and Vakil [9] observed that about 18 % of the hybridomas they produced from foetal or neonatal liver showed anti-idiotypic or anti-antiidiotypic activity. In addition, many of these antibodies have multiple specificities. That these naturally occurring autoreactive antibodies are produced by Ly-1 B cells is strongly suggested by the fact that, in each case, these hybridomas are derived from tissues in which the majority of the B cells are of the Ly-1 B phenotype. It appears that Ly-1 B cells are intimately involved in the immune system's knowledge of self, possibly acting as a repository of neonatal immunological experiences. Ly-1 B cells are good candidates to fill such a function, since they appear early in B-cell development and are maintained as a stable population for the life of the animal.

3) Ly-1 B cells have a restricted ability to respond to exogenous antigens.

In direct contrast to their reactivity with autoantigens, Ly-1 B cells have proven unable to respond to most «classical» exogenous T-dependent and T-independent antigens. In both NZB and

BALB/c mice, antibody responses to SRBC, TNP-KLH, TNP-Ficoll and TNP-LPS were all found among Ly-1⁺ splenic B cells [7]. The only antigen to which we have been able to identify a Ly-1 B response is phosphorylcholine-KLH (PC-KLH) (AMS, unpublished results). However, Marcolino *et al.* have shown that the CH series of Ly-1 B lymphomas, in fact, are specific for phosphatidylcholine exposed on BrmRBC [5]. Thus, the anti-PC activity of Ly-1 B cells may be yet another autoimmune response. One interesting possibility is that this dichotomy between the antigenic repertoire of Ly-1⁺ and Ly-1⁻ B cells might reflect a differential utilization of V_H gene families by the two B-cell lineages [11].

4) Ly-1 B cell involvement in autoimmune disease.

Given the propensity of Ly-1 B cells to produce autoantibodies in normal animals, it is not surprising to find that the level of these cells is elevated in strains of mice which serve as models for autoimmune disease. In NZB mice, 100 % of the peritoneal and 10-15 % of the splenic B cells are Ly-1⁺ [1]. It is these cells which produce a significant portion of the autoantibodies present in

these mice. In motheaten mice, which show a particularly severe autoimmune syndrome, all of the B cells are of the Ly-1 B phenotype [12]. Sidman *et al.* have shown that these Ly-1 B cells constitutively produce a factor which induces B-cell maturation [13]. In humans, a similar association has been seen in rheumatoid arthritis. The number of Leu-1 B cells in the blood of patients with rheumatoid arthritis was increased 20-fold in comparison with healthy controls. However, the level of Leu-1 B cells in individual patients did not correlate with clinical disease activity [14]. Thus, while there is a clear association between Ly-1 B cells and a number of autoimmune diseases, the cause and effect relationship between Ly-1 B cells and autoimmune dysfunction remains uncertain.

From the data presented in this paper, it is clear that, although Ly-1 B cells represent a relatively small fraction of the B-lymphocyte population, they play a very large role in the production of autoantibodies, both in normal and autoimmune animals. While we have accumulated a great deal of information since their initial identification, the function of Ly-1 B cells and the autoantibodies that they produce certainly remains a fertile field for future research.

References.

- [1] HAYAKAWA, K., HARDY, R.R., PARKS, D.R. & HERZENBERG, L.A., The «Ly-1 B» cell subpopulation in normal, immunodeficient, and autoimmune mice. *J. exp. Med.*, 1983, **157**, 202.
- [2] HAYAKAWA, K., HARDY, R.R. & HERZENBERG, L.A., Peritoneal Ly-1 B cells: genetic control, autoantibody production, increased lambda light chain production. *Europ. J. Immunol.*, 1986 (in press).
- [3] DAVIDSON, W.F., FREDERICKSON, T.N., RUDIKOFF, E.K., COFFMAN, R.L., HARTLEY, J.W. & MORSE, W.C. III., A unique series of lymphomas related to the Ly-1⁺ lineage of B-lymphocyte differentiation. *J. Immunol.*, 1984, **133**, 744.
- [4] HARDY, R.R., HAYAKAWA, K., HERZENBERG, L.A., MORSE, H.C., III, DAVIDSON, W.F. & HERZENBERG, L.A., Ly-1 as differentiation antigen on normal and neoplastic B cells. *Curr. Top. Microbiol. Immunol.*, 1984, **113**, 231.
- [5] MARCOLINO, T.J., ARNOLD, L.W. & HAUGHTON, G., Phosphatidylcholine is recognized by a series of Ly-1⁺ murine B-cell lymphomas specific for erythrocyte membranes. *J. exp. Med.*, 1986, **163**, 155.

- [6] HAYAKAWA, K., HARDY, R.R., HERZENBERG, L.A. & HERZENBERG, L.A., Progenitors for Ly-1 B cells are distinct from progenitors for other B cells. *J. exp. Med.*, 1985, **161**, 1554.
- [7] HAYAKAWA, K., HARDY, R.R., HONDA, M., HERZENBERG, L.A., STEINBERG, A.D. & HERZENBERG, L.A., Ly-1 B cells: Functionally distinct lymphocytes that secrete IgM autoantibodies. *Proc. nat. Acad. Sci. (Wash.)*, 1984, **81**, 2494.
- [8] BOFILL, M., JANOSSY, G., JANOSSA, M., BURFORD, G.D., SEYMOUR, G.J., WERNET, P. & KELEMEN, E., Human B-cell development. — II. Subpopulations in the human fetus. *J. Immunol.*, 1985, **134**, 1531.
- [9] KEARNEY, J. & VAKIL, M., Functional idiotype networks during B-cell ontogeny. *Ann. Inst. Pasteur/Immunol.*, 1986, **137 C**, 77.
- [10] HOLMBERG, D., WENNERSTROM, G., ANDRADE, L. & COUTINHO, A., The high idiotypic connectivity of «natural» newborn antibodies is not found in adult mitogen-induced B-cell repertoires. *Europ. J. Immunol.* (in press).
- [11] YANCOPOULOS, G.D., DESIDERIO, S.V., PASKIND, M., KEARNEY, J.F., BALTIMORE, D. & ALT, F.W., Preferential utilization of the J_H-proximal V_H segments in pre-B-cell lines. *Nature (Lond.)*, 1984, **311**, 727.
- [12] SIDMAN, C.L., SHULTZ, L.D., HARDY, R.R., HAYAKAWA, K. & HERZENBERG, L.A., Preferential production of specific Ig isotypes by Ly-1(+) B cells in viable motheaten and normal mice. *Science*, 1986 (submitted).
- [13] SIDMAN, C.L., MARSHALL, J.D., MASIELO, N.C., ROTHS, J.B. & SHULTZ, L.D., Novel B-cell maturation factor from spontaneously autoimmune viable motheaten mice. *Proc. nat. Acad. Sci. (Wash.)*, 1984, **81**, 7199.
- [14] PLATER-ZYBERK, C., MAINI, R.N., LAM, K., KENNEDY, T.D. & JANOSSY, G., A rheumatoid arthritis B-cell subset expresses a phenotype similar to that in chronic lymphocytic leukemia. *Arthritis Rheum.*, 1985, **28**, 971.

DISCUSSION

S. Avrameas:

Objectivity in the vast area of autoimmunity, where scientific knowledge is partial, incomplete and diffuse, relies on subjectivity. Therefore, my comments, because they are based on my subjectivity, *i.e.* my knowledge and my truth, cannot be objective.

R. Schwartz expresses views and hypotheses which are close to mine. I agree that there are formidable problems to solve before we can accept the relevance of natural autoantibodies, but I would also argue that the same holds true for Ehrlich's and Burnet's concept of immunity although, based on their views, an immense sum of work has already been accomplished. It is correct that, in mice, all or nearly all polyspecific antibodies are of the IgM isotype, but autoreactive human paraproteins with polyspecific antibody function

belong to any of the IgM, IgA and IgG isotypes. Therefore, the polyspecificity of natural antibodies is preferentially but not exclusively associated with the IgM isotype. It is correct that ELISA has been used for most of the studies with natural antibodies but, in addition, almost equally often, immunocytochemical procedures were also employed. Both ELISA and immunocytochemical procedures are making use of antigens immobilized on solid phases and it is possible, as suggested by Dr Schwartz, that this kind of interaction gives results which can be different from those obtained with procedures based on a liquid phase antigen-antibody reaction. I think, however, that the interaction of an antibody with an immobilized antigen would be more representative of what one would expect to happen in nature. In that sense, considering that the immense majority of investigators,

maybe with a few notable exceptions, are not working carelessly, I would have the tendency to place more trust in the results obtained with solid phase procedures. Finally, even if the hypothesis advanced by Dr Schwartz is correct, *i.e.* that autoantibodies are not preserved by evolutionary forces but rather by the molecular mechanism which generates immunoglobulin diversity, one cannot exclude that natural autoantibodies do play an important role in the immune system.

There is a line of evidence in the paper presented by Dr Stall and collaborators indicating that the Ly-1 B lineage may correspond to the autoreactive B cells producing the natural autoantibodies. There are in addition, however, results that do not favour this hypothesis. Thus, in contrast to the low number (1-2 %) of Ly-1 B found in spleen, fusion experiments performed with adult mouse spleen cells have shown that, depending on the immunological status and species of the mouse as well as the fusion myeloma partner, there is a high number of splenocytes producing natural autoantibodies. Furthermore, there is no evidence, such as that reported for Ly-1 B cells, indicating a correlation between natural autoantibody producing cells and autoimmune diseases.

I would agree with the conclusion of Dr Davidson and collaborators that IgG autoantibodies are more pathogenic and that they differ from the germline-encoded autoantibodies. For the reasons I have discussed in my paper, I do not feel, however, that the increased pathogenicity of IgG, as compared to that of the IgM encoded by the germline, is due to its higher affinity for the antigen, but rather to its strict specificity. The fact that pathogenic IgG can be produced by somatic mutations seems more than probable. I think, however, that such mutations occur more frequently in gene families derived from the initial germline genes than in the germline genes themselves. Finally, the experiments described by Dr Davidson and collaborators with antibacterial (anti-phosphorylcholine) and autoreactive (anti-phosphorylcholine + anti-double-stranded DNA) anti-

bodies are representative of situations which the immune system often has to face. Phosphorylcholine determinants as well as DNA are present in both mice and bacteria, and can be considered equally well as self and non-self. One may ask how this subtle distinction can be made by the immune system of the mouse without a precise knowledge of what is self.

Drs Bach and Jacob, when considering the specificity of a monoclonal anti-DNA antibody which also reacts with cell-surface proteins they have isolated and studied, raise the question of the triggering antigen in SLE and the pathogenic role of the anti-DNA antibodies. Compared to IgM murine monoclonal polyspecific natural autoantibodies which react with DNA, cytoskeletal and other proteins, the above monoclonal antibody is of the IgG isotype and appears to possess a more restricted polyspecificity. Assuming the validity of the hypothesis that cells carrying the polyspecific natural autoantibodies as receptors after stimulation by a given antigen (non-self or altered self) will proliferate to cells producing antibodies more specific for that antigen, I would consider that the monoclonal anti-DNA antibody of Drs Bach and Jacob is the consequence of this mechanism, and hence that it might play a more pathogenic role than natural polyspecific autoantibodies reacting with DNA.

I really do not feel competent to discuss the papers of Dr Lambert and collaborators and Dr Todd and collaborators. In any case, I could not find any fundamental objection that could challenge their conclusions.

In conclusion, I find that the studies presented in this Forum show, firstly, that autoantibodies can be present in humans and mice without necessarily being accompanied by a pathological manifestation and secondly, that these autoantibodies can be generated following various pathways.

P.H. Lambert:

I would like to suggest one short comment for A.M. Stall. A main fea-

ture of SLE and of experimental models of SLE is the production of anti-DNA antibodies belonging to T-dependent subclass of IgG. Since Ly-1B cells are IgM producers, one may wonder whether they can account for the bulk of anti-DNA antibodies produced in these conditions.

J.F. Bach:

The set of papers presented in this Forum reaches a consensus of the existence of natural autoantibodies probably coded for by germline genes. The interesting concept initially put forward by S. Avrameas of plurispecific receptor-bearing clones manufacturing such antibodies and leading, by somatic mutations, to conventional antibody-forming clones is attractive. As a corollary of these hypotheses, one must assume with B. Diamond that two types of autoantibodies exist. The first one is IgM, polyspecific and encoded by germline genes; the other is IgG, monospecific and encoded by rearranged gene. In this scheme, the second category of autoantibody is in no way different from antibodies produced against conventional antigens. It is likely that pathogenic autoantibodies, usually of the IgG class and of high affinity, belong to the second class, although S. Avrameas reports a high affinity for some natural autoantibodies of the first type and B. Diamond notes that some of these antibodies may be IgG in man. It is logical that the physiologic type of autoantibodies will more often express recurrent idiotypes than the hyperimmune type, which probably explains why, in our studies, we have essentially found private idiotypes on our IgG monoclonal autoantibodies strictly directed against double-stranded DNA which are presumably of the second type [1, 2].

In other words, autoimmunity should be considered in two different settings: *physiologic* autoimmunity, present in normal subjects during the whole life span and potentially playing a major role in the initiation of B-cell differentiation; and *pathologic* autoimmunity, close to conventional immu-

nity. Ly1⁺ B cells would essentially produce the physiologic autoantibodies before maturing into Ly1⁻ B cells that produce the conventional antibodies or hyperimmune type autoantibodies. The report by the Herzenbergs that Ly1⁺ B cells produce presumably pathogenic autoantibodies in lupus mice argues against these hypotheses, but were the autoantibodies in the studies referred to truly pathogenic autoantibodies?

The problem remains of the triggering mechanism of pathogenic hyperimmune autoantibody formation. The presence of the autoantigen is probably necessary, as suggested by the elegant experiment by Roitt's group showing that neonatal thyroidectomy prevents the occurrence of antithyroglobulin autoantibodies in the obese strain of chicken [3]. The aberrant MHC antigen expression described by Bottazzo provides a satisfactory explanation in many regards, particularly for the clustering of autoantibodies showing specificity for a defined organ. The absence of aberrant MHC antigen expression observed on the islet cells of spontaneously diabetic BB rats (Savino, W., Timsit, J. and Bach, J.F., unpublished results) and NOD mice [4] at the time of onset of the disease (which cannot be studied in man) urges for caution before claiming that aberrant MHC antigen expression is primitive and not secondary to the disease; however, more studies are needed before fully appreciating the experimental diabetes data. In fact, Todd's scheme could also apply to SLE if the membrane protein that we have described stimulates the production of antibodies with secondary cross-reactions with DNA. Since many cell types express the protein in their membrane, it is likely, however, as suggested by Todd *et al.*, that the aberrant MHC antigen expression is probably exerted at the macrophage level. Note that, in particular settings, as discussed by Lambert, other stimuli may intervene, such as polyclonal activation, as provided by the allogeneic reactions, and cross-reacting antigens, as represented by bacteria.

Finally, one major problem remains unsolved concerning the pathogenicity

of autoantibodies. Why are the physiologic autoantibodies not pathogenic? We have seen that the affinity explanation may not be valid. It is also not obvious that insufficient concentrations are the correct explanation. More generally, since only hyperimmune type

autoantibodies induce clinical manifestations, one may tentatively assume that among the whole family of autoantibodies specific for a given molecule, only a few will recognize (probably at random) a crucial epitope leading to functional lesions or cytolysis.

References.

- [1] TRON, F., LEGUERN, C., CAZENAVE, P.A. & BACH, J.F., Intrastrain recurrent idiotypes among anti-DNA antibodies of (NZB X NZW) F₁ hybrid mice. *Europ. J. Immunol.*, 1982, **12**, 761-766.
- [2] TRON, F., JACOB, L. & BACH, J.F., Murine monoclonal anti-DNA antibodies with an absolute specificity for DNA have a large amount of idiotype diversity. *Proc. nat. Acad. Sci. (Wash.)*, 1983, **80**, 6024-6027.
- [3] PONTES DE CARVALHO, L.C., TEMPLEMAN, J., WICK, G. & ROITT, I.M., The role of self-antigen in the development of autoimmunity in obese strain chickens with spontaneous autoallergic thyroiditis. *J. exp. Med.*, 1982, **15**, 1255.
- [4] MIYAZAKI, A., HANAFUSA, T., YAMADA, K., MIYAGAWA, J., NAKAJIMA, H., NONAKA, K. & TARUIS, S., Predominance of T lymphocytes in pancreatic islets and spleen of pre-diabetic non-obese diabetic (NOD) mice: a longitudinal study. *Clin. exp. Immunol.*, 1985, **60**, 622-630.

G.F. Bottazzo and the London group's arguments and reply:

In 1986, the concept of self-perception by the immune system is widely accepted, the well known examples being recognition of antigen in the context of self-MHC molecules, and the accumulating evidence for idiotypic/antiidiotypic interactions.

The existence of natural autoantibodies, discussed by several of the participants in the Forum, is therefore not a great surprise although, as pointed out by Bob Schwartz, the validity of the observations requires rigorous verification. An important question also raised by Schwartz, but not directly addressed, is the relationship, if any, between these antibodies present in normal animals and individuals, and those associated with autoimmune diseases. We feel that the available evidence does not favour a strong relationship of this type. First of all, natural autoantibodies are mainly IgM, are present in the serum

at low titres and are very rarely detected by conventional autoantibody tests, whereas disease-related autoantibodies are almost entirely IgG and can reach very high titres in the circulation.

However, it may be important in this context to make a distinction between organ-specific and non-specific autoimmune diseases. It is remarkable that most of the participants in the Forum concentrate on organ non-specific autoantibodies, particularly anti-DNA antibodies, as found in SLE: indeed, in this instance, it is feasible that the relationship does exist between the disease-related autoantibodies and natural anti-DNA antibodies. For example, Jean-François Bach and Laurent Jacob discuss the evidence that anti-DNA antibodies of SLE are cross-reactive with other cellular factors, and the characteristic of many natural autoantibodies is their cross-reactivity with a variety of molecules. This could be related to the low «intrinsic affinities» and idiotypic similarities of the latter antibodies, des-

cribed by Stratis Avrameas. By contrast, the situation is very different in organ-specific autoimmune diseases, where the autoantibodies specifically recognize autoantigens which are unique to the target organ: examples are the thyroid microsomal antigen (TMAg, MW 105 Kd) and the 64-Kd antigen of pancreatic B cells.

At the cellular level, Alan Stall and associates discuss the role of Ly-1/Leu⁺ B cells in synthesizing natural autoantibodies, and point out that these cells are increased in mice predisposed towards immunity. Again, however, the diseases which develop in these animals are of the SLE type. By contrast, although Leu1⁺ B cells are raised in patients with rheumatoid arthritis (which can be considered to have «intermediate organ specificity»), this phenomenon did not correlate with disease activity. It is also remarkable how difficult it is proving to be to produce human hybridomas synthesizing organ-specific autoantibodies as compared with autoantibodies recognizing multiple organs, despite the efforts of many investigators. For example, Notkins' group fused peripheral blood lymphocytes from diabetic patients and readily obtained hybridomas of the latter type, but none of the former. These antibodies were also detected by fusing lymphocytes from normal individuals and recognize a common antigen with a MW (35 Kd) distinct from that of organ-specific autoantigens. Again we are aware of no reports of hybridomas against autologous TMAg, whereas a number of groups have produced monoclonal antibodies to thyroglobulin (Tg) which may be considered a different type of autoantigen from TMAg (*vide infra*). The reasons for these differences in ease of hybridoma production are unclear, but could be related to the tissue distribution of organ-specific compared with non-specific autoreactive B cells (with the former more restricted to the target tissues?), or differences in their differentiation (*e.g.* IgG-producing and IgM-producing cells, respectively?) or activation states.

A closer look at the antigens recognized by natural and organ-specific autoantibodies also highlights the dis-

tinction: the molecules recognized by natural autoantibodies are mainly located either intracellularly (*e.g.* nucleic acids, tubulin, actin, etc.) or are secreted products (*e.g.* transferrin, albumin, collagen, etc.). Can we therefore consider them harmful? By contrast, the antigens which appear to be most important in organ-specific autoimmunity are cell-specific and integral molecules of the plasma membrane, where they are recognized by cytotoxic antibodies, *e.g.* anti-TMAg and islet cell surface antibodies. Whether the same is true of organ non-specific diseases, as suggested by Bach and Jacob, remains a possibility. However, in their example, the surface molecules recognized by anti-DNA antibodies are neither organ- nor cell-specific and, interestingly, precipitate a molecule very similar in size (34 Kd) to that recognized by Notkins' hybridoma antibodies of pseudo-organ-specific reactivity, as discussed above.

In this context, the differences between the classical thyroid antigens, TMAg and Tg, mentioned above in relation to hybridoma production, are again apparent: thus, Tg, which is recognized by some natural autoantibodies, is the converse of TMAg in that it is present in the circulation but difficult to detect at the surface of thyroid epithelial cells. Furthermore, immunizing animals with human Tg readily raises an antibody response, whereas solubilized TMAg is notorious for its very low immunogenicity, and the mouse monoclonal anti-human TMAg which has been produced so far recognizes epitopes close to, but distinct from, those seen by autoantibodies.

It is certainly true that apparently normal individuals (mostly female, known to be predisposed to autoimmunity) can possess circulating anti-TMAg, but this corresponds with a relatively high incidence of subclinical focal thyroiditis with which thyrocyte MHC class II expression appears to be associated. This brings us back to our point that the target organ may play a major role in promoting the development of organ-specific autoantibodies, a mechanism which we believe does not apply to the formation of naturally occurring autoantibodies. By contrast, a systemic

stimulation, as in the chimaeric situation described by Lambert and his associates, leads to the expansion of clones producing organ non-specific antibodies to DNA.

For the present, therefore, we shall keep an open mind on the relationship

between natural and what might be termed «unnatural» (*i.e.* pathological) autoantibodies. However, we do feel that breaching the gap between these entities will be a major construction project, which may still have to be abandoned in mid-stream.

The work cited in this reply, which is additional to that referenced in our contribution to the Forum, is fully reviewed and discussed in the following articles.

References.

- TODD, I. & BOTTAZZO, G.F., Laboratory investigation of autoimmune endocrine diseases, in «Laboratory investigation of immunological disorders», Clinics in immunology and allergy (R.A. Thompson) vol. V (pp. 613-638). W.B. Saunders Co., London, 1985.
- BOTTAZZO, G.F., PUJOL-BORRELL, R.D., GALE, E., Etiology of diabetes, the role of autoimmune mechanisms, in «The Diabetes Annual/1» (K.G.M.M. Alberti & L.P. Krall) (p. 1652). Elsevier, Amsterdam, 1985.
- BOTTAZZO, G.F., PUJOL-BORRELL, R.D., GALE, E., Autoimmunity and diabetes: progress, consolidation and controversy, in «The Diabetes Annual/2» (K.G.M.M. Alberti & L.P. Krall). Elsevier, Amsterdam, 1986 (in press).

Dr. Stall's comments:

Both Schwartz and Avrameas suggest that preservation of autoreactivity may be a consequence and/or requirement of a functional immune system. It is not unreasonable to expect that a specialized lineage or subset of B cells would be devoted to carrying out this function. We would again suggest that Ly-1 B cells are a prime candidate for the preservation of this «knowledge of self». It is interesting to note that in the studies of Lambert *et al.*, the anti-ssDNA antibodies were produced by the donor cells. Given the ability of Ly-1 B cells to repopulate following adoptive transfer, the possibility that they are, in fact, the producers of this anti-ssDNA warrants investigation. This is the main point that we would like to make; while Ly-1 B cells may not be involved in the production of all autoantibodies, they should be considered when questions of «naturally occurring» or autoantibodies arise.

R.S. Schwartz:

It appears that Avrameas, Diamond and I are, so to speak, playing in the same string trio. And even though our musical themes are slightly different, a fundamental sense of harmony emerges from the notes. Avrameas may be compared to the cellist whose continuo provides the chords which set the rhythmic beat for the variations played by Diamond's violin and my viola's counterpoint. Avrameas' part is called «natural autoantibodies», Diamond's instrument sings of mutations, and I fiddle pathogenic autoantibodies. The name of the piece we are playing is «V-gene diversification». The cello, appropriately enough, defines the background; *i.e.*, the pre-immune repertoire consists, at least in part, of autoantibodies. And it is from these natural autoantibodies that the variants induced by contact with foreign antigens arise. The violin's notes weave between autoantibodies and antibacterial anti-

bodies: some autoantibodies may be variants of antibodies against foreign antigens. Why not? V-gene mutation is, like all mutations, a random process (or, musically speaking, *aleatory*), so Diamond's proposal is not only a formal possibility, but one backed by experimental evidence. The viola player may seem to be reading the notes upside down, but the music belongs to the same trio: pathogenic autoantibodies arise by expansion of a pool of natural autoantibody-producing precursors.

The viola's notes are written in idiotypes and amino acid sequences, and all of them return, ultimately, to the cello's continuo: germline V genes. There are, however, other tunes which our trio has not played: antigenic mimicry, idiotypic networks and the «bad luck» of having inherited the wrong MHC structures are also important parts of the repertoire. Expanding the trio into a coherent symphony may require considerable talent if cacophony is to be avoided.

MOTS-CLÉS: Autoanticorps, Immunogenèse, Immunotolérance; Forum.