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Self-reactive antibodies (natural autoantibodies) in healthy individuals

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Abstract

Antibodies that are present in the serum of healthy individuals in the absence of deliberate immunization with any antigen, are refered to as natural antibodies. A vast majority of natural antibodies react with one or more self antigens and are termed as natural autoantibodies. The importance of natural autoantibodies in immune regulation has long been neglected, since tolerance to self was thought to be primarily dependent on the deletion of autoreactive clones, rather than on peripheral suppressive mechanisms. Clonal deletion and energy cannot account, however, for the prevalence of natural autoreactivity among healthy individuals. It is now well established that autoreactive antibodies and B cells, and autoreactive T cells, are present in healthy individuals, and in virtually all vertebrate species. Autoreactive repertoires are predominantly selected early in ontogeny. Questions pertaining to the role of natural antibodies in the regulation of the immune response and maintenance of immune homeostasis and to the distinction between natural autoreactivity and pathological autoimmunity have not been adequately addressed. Here, we focus on the current knowledge on the physicochemical and functional properties of NAA in man, and the use of NAA for therapeutic intervention.

Keywords: Natural autoantibodies; Autoreactivity; Repertoires; Immunoglobulins

1. Introduction

Natural antibodies refer to antibodies that are present in the serum of healthy individuals in the absence of deliberate immunization with the target antigen (Coutinho et al., 1995). Autoantibodies are immunoglobulins that react with at least one self antigen, whether they originate from healthy individuals or patients with autoimmune disease. The importance of natural antibodies reactive with self antigens (natural autoantibodies, NAA) has long been neglected, as tolerance to self was thought to be primarily dependent on the deletion of autoreactive clones during ontogeny. It is now well established, how-

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ABR: NAA, natural autoantibodies; CDR, Complementarity determining region; V gene, variable gene; Tg, thyroglobulin; FVIII, factor VIII; SLE, systemic lupus erythematosus; IVIg, intravenous immunoglobulin; Tg, thyroglobulin.

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ever, that autoreactive antibodies and B cells, and autoreactive T cells, are present in healthy individuals, and that autoreactive repertoires are predominantly selected during fetal life (Coutinho and Kazatchkine, 1994). Since the first report by Boyden (Boyden, 1965), NAA have been found in virtually all vertebrate species (Avrameas, 1991).

The present review focusses on NAA in normal human serum. It summarizes the current knowledge on the physicochemical and functional properties of NAA, the genes encoding NAA, their significance as a part, together with autoreactive T cells, of the autoreactive compartment of the normal immune system, and the use of NAA for therapeutic intervention.

2. Characteristics of natural autoantibodies

2.1. Isotype

NAA belong to the IgM, IgG and IgA isotypes (Table 1). It was initially considered that NAA are preferentially of the IgM isotype, since NAA were first reported in mice where IgM predominates over the other isotypes at the neonatal stage. Thus, cord blood IgM in mouse (Holmberg et al., 1986a) and man (Kearney and Vakil, 1986), is predominantly, if not exclusively, self-reactive. Most of the NAA in adult serum is of the IgG class (Avrameas, 1991). The mechanisms underlying the isotype switch from μ-chain to γ-chain for natural autoantibodies are unclear at present, particularly with regard to the mechanisms involved in the processing and presentation of self antigens (including idiotopes) to T cells. Natural autoreactive T cells contribute in a large measure to the selection of natural autoreactive B-cell repertoires under physiological conditions (Huetz et al., 1988; Lymberi et al., 1989). Autoreactive $CD4^+$ T lymphocytes specific for a number of self antigens, including myelin basic protein (Martin et al., 1990), the acetylcholine receptor (Moiola et al., 1994; Elson and Williams, 1995), the thyroglobulin-stimulating hormone receptor (Kellerman et al., 1995) and the gpIIbIIIa platelet antigen (Filion et al., 1995) have been reported in healthy individuals. It could be speculated, alternatively, that natural autoreactive B cells are endowed with some switching ability in the absence of cognate interactions with T cells, a hypothesis consistent with the finding of small amounts of IgG in the serum of CD40L-deficient patients with the hyper-IgM syndrome (Durandy et al., 1993). Finally, self-reactive IgG in adult serum could originate, in part, from natural autoreactive B cells encountering epitopes on the surface of invading agents ('foreign antigens') that are cross-reactive with self-epitopes. As discussed below, the latter hypothesis cannot account for the observed restricted and conserved nature of natural autoreactive IgG antibody repertoires (Mouthon et al., 1995a,b).

Table 1
Characteristics of natural autoantibodies

IgM, IgG, IgA isotypes
Affinity for self antigen ranging between 10^{-5} and 10^{-8} M
High polyreactivity
High connectivity
Encoded by germ-line V genes
Restricted repertoire of antibody reactivities

2.2. Polyreactivity

NAA specific for one self antigen have been reported in normal serum (Lutz and Wipf, 1982; Galili et al., 1987; Bendtzen et al., 1990). Most of the NAA characterised so far are, however, polyreactive in that the antibodies are capable of recognizing several self and 'foreign' antigens in mice (Dighiero et al., 1983; Prabhakar et al., 1984; Dighiero et al., 1986; Rossi et al., 1990) and in man (Elson et al., 1979; Rossi et al., 1990). Depending on their specificity, natural IgG autoantibodies which have been affinity-purified from human serum exhibit similar or higher degrees of polyreactivity as compared with autoantibodies from patients with autoimmune diseases (Hurez et al., 1993a). Experiments using site-directed mutagenesis have indicated that the complementarity determining region (CDR) 3 in the V_H domain is primarily involved in determining the relative polyreactivity of NAA (Ichiyoshi and Casali, 1994; Martin et al., 1994). Polyreactivity of NAA toward self antigens does not correlate with their connectivity, i.e. their ability to interact with

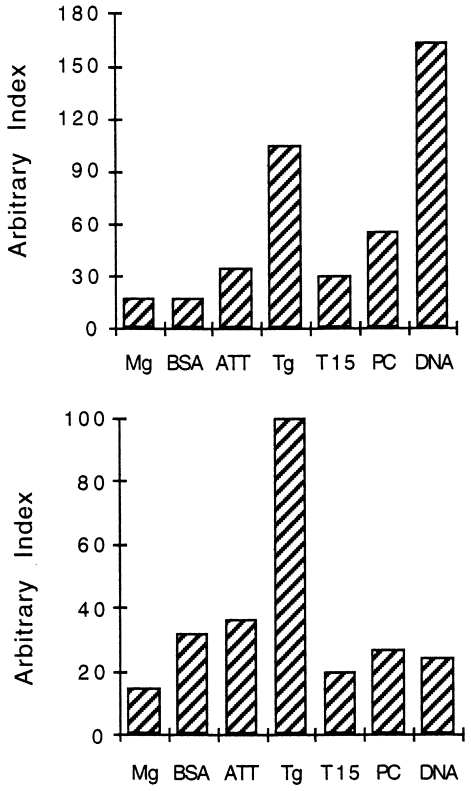

Fig. 1. Polyreactivity of natural anti-thyroglobulin (Tg) IgG autoantibodies. The fraction of normal IgG that exhibits high connectivity was purified from IVIg (intravenous immunoglobulin) as the acid-eluate of an affinity chromatography of IVIg on Sepharose-bound $F(ab')_2$ fragments of IVIg. Anti-Tg IgG were then purified by affinity chromatography on Sepharose-bound human Tg from both the unfractionated IVIg preparation (upper panel) and the 'connected' fraction of IVIg (lower panel). Antibody reactivity was tested on a panel of antigens including the autoantigens myoglobin (Mg), DNA, phosphorylcholine (PC), Tg, the peptide T15H(50–73) and the non-self antigens bovine serum albumin (BSA) and tetanus toxoid (ATT) using 20 μg/ml of IgG in all assays. The results were expressed relative to the reactivity of IgG with Tg, arbitrarily given a value of 100 (modified from Hurez et al., 1993a).

variable regions of other autoantibodies (Rossi et al., 1990; Hurez et al., 1993) (Fig. 1). Polyreactivity does not mean lack of specificity: thus, each polyreactive NAA encompasses its own distinct set of epitopic specificities and therefore is unique (Ternynck and Avrameas, 1986). Indeed, we have recently observed that monoclonal IgM from patients with Waldenström's macroglobulinemia may recognize none of the antigens present in a tissue protein extract or may recognize as many as 30 different protein bands in the same extract (Lacroix-Desmazes et al., 1997). Although no particular role for polyreactivity has been documented, it has been proposed that polyreactivity of NAA may represent a selective advantage. In this respect, it is helpful to envisage the antigen–antibody interaction as representing the interaction of two complementary structures rather than a 'uni-directional' reaction of a paratope with a 'target' epitope and the variable region of immunoglobulins as expressing several distinct interacting sites (i.e. paratope and idiotopes) (Jerne, 1984; Kohler et al., 1988).

2.3. Affinity

Early publications reported NAA as exhibiting low affinities and high avidities for self antigens (Nakamura et al., 1986; Ternynck and Avrameas, 1986; Casali and Notkins, 1989). In fact, the literature suggests that NAA may exhibit a broad range of affinities, with dissociation constants ranging from 10^{-5} to 10^{-8} M (Casali and Notkins, 1989b; Bendtzen et al., 1990; Avrameas, 1991; Adib-Conquy et al., 1993; Diaw et al., 1997). NAA specific for IL-1α were shown to express a dissociation constant of less than 5×10^{-11} M (Svenson et al., 1990).

The availability of novel technologies to measure protein–protein interactions should provide new information that may be useful for the understanding of the physiological relevance of the interactions of NAA with self antigens. Thus, using the BIAcore® technology, we have observed an overall affinity of natural IgG autoantibodies specific for molecules such as HLA class I, CD4, the RGD motive and autologous blood group antigens, in the micromolar range (Fig. 2) (Hurez et al., 1994; Kaveri et al., 1996; Spalter et al., 1997; Vassilev et al., 1997).

The notion that an antibody has to be of high affinity in order to be biologically relevant originates primarily from the analysis of the requirements for an efficient immune response against pathogens. This concept does not necessarily apply to natural antibodies. Thus, network interac-

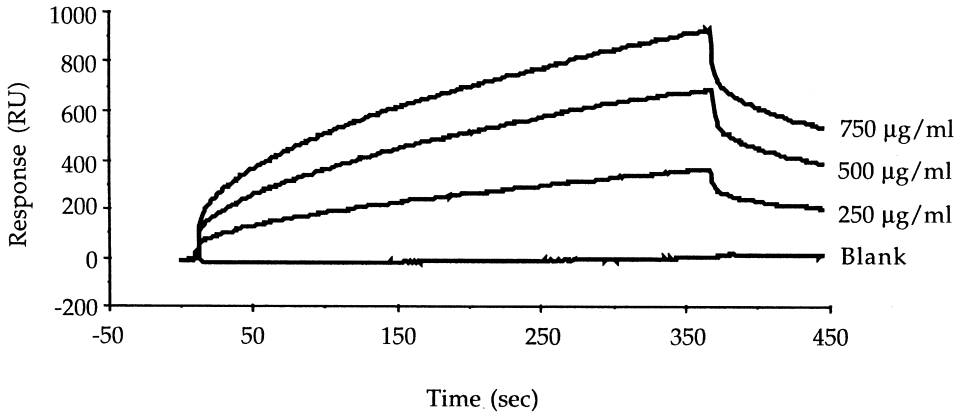

Fig. 2. Real-time measurement of complex formation between normal human IgG (IVIg) and the B07.75–84 peptide derived from HLA class I molecules. The figure shows an overlay plot of the sensorgrams obtained following injection of three concentrations of affinity-purified anti-peptide $F(ab')_2$ fragments of IVIg ranging from 250 to 750 $\mu g/ml$. The decrease in the signal at the end of the injection corresponds to the dissociation of the non-covalently formed complexes. The bottom curve depicts the injection of 750 $\mu g/ml$ of $F(ab')_2$ fragments of IVIg onto control, uncoupled dextran matrix (modified from Kaveri et al., 1996).

tions of low affinity antibodies may result in novel biological properties that emerge from the organization of the network and may not be predicted from the biological activity of the individual components of the network. In addition, the monovalent binding reaction of an antibody can be of low affinity whereas multivalent binding results in high avidity (Avrameas and Ternynck, 1995) and the biological relevance of an antigen–antibody interaction is dependent on the local concentration of antigen and antibody as much as on the mere binding characteristics of the antibody molecule.

2.4. Connectivity

Connectivity characterizes the degree to which variable (V) regions of antibodies interact with complementary V regions in the immunoglobulin fraction of the serum of an individual and complementary V regions of antigen receptors on lymphocytes. NAA have been shown to express higher degrees of connectivity than 'immune' antibodies generated by deliberate immunization with a 'foreign' antigen.

3. Cells producing NAA and genes encoding natural autoantibodies

3.1. Cells producing NAA

The evidence that NAA originate from a distinct subset of human B cells remains controversial. Murine B lymphocytes have been classified on the basis of their location and expression of phenotypic markers into conventional $CD5^-$ B cells (B-2 cells), peritoneal $CD5^+$ B lymphocytes (B-1a cells) and peritoneal $CD5^-$ B lymphocytes (B-1b cells). In the mouse, the repertoire of B-1 $CD5^+$ cells is comprised predominantly of antibodies that react with self antigens (Bikah et al., 1996). In the human fetus, at a time when the expressed B cell repertoire is primarily autoreactive (Kearney and Vakil, 1986), $CD5^+$ B cells represent 50–70% of the B cells in spleen (Antin et al., 1986; Hardy et al., 1987) and more than 90% of B cells in cord blood (Durandy et al., 1990) and in liver (Casali and Notkins, 1989a; Kipps, 1989). EBV-transformation of peripheral blood B cells of healthy donors expressing the CD5 marker, results in the secretion of polyreactive autoantibodies (Nakamura et al., 1988; Casali and Notkins, 1989). Increased numbers of circulating $CD5^+$ B cells have been reported in patients with rheumatoid arthritis and Sjögren's syndrome, in whom these cells were suggested to produce high affinity autoantibodies (Burastero et al., 1988; Youinou et al., 1988). However, $CD5^-$ B cells have also been shown to produce polyreactive NAA (Kasaian et al., 1992). Furthermore, several lines of evidence argue against differences between natural and disease-associated autoantibodies secreted by $CD5^+$ and $CD5^-$ B cells, with

regard to the kinetics of production, the primary amino acid sequences in the CDRs and the extent of polyreactivity (Araga et al., 1995; Chen et al., 1995; Kiyoi et al., 1995; Maloum et al., 1995; Ye et al., 1996). Based on the available data on the selection of B cell repertoires in the mouse (Freitas et al., 1991; Marcos et al., 1991; Sundblad et al., 1991), we consider that NAA may be produced by most B cells leaving the bone marrow that become 'naturally' activated, be it by an autoantigen or by an idiotypically-complementary V region of an antigen receptor. In newborn and in adult germ-free mice, naturally activated B cells represent approx. 5–15% of the total number of splenic B lymphocytes (Holmberg et al., 1986b). As discussed in the next paragraph, it may be that some of these cells producing polyreactive NAA, undergo affinity maturation into high affinity antibodies to foreign antigens or pathogenic high-affinity antibodies to self antigens, irrespective of CD5 expression.

3.2. Genes encoding NAA

V gene utilization in the early stages of development is not random: thus, non-stochastic V_H gene expression has been observed in newborn mice at a time when NAA predominate (Reth et al., 1986; Jeong and Teale, 1988, 1989; Malynn et al., 1990). In a similar fashion, the smallest and most DJ_H-proximal of the seven human gene families, V_H5 and V_H6, were shown to predominate during human fetal life and in the newborn (Alt et al., 1987; Schroeder et al., 1987; Cuisinier et al., 1989; Nickerson et al., 1989; Schroeder and Wang, 1990; Berman et al., 1991; Raaphorst et al., 1992). V_H genes preferentially expressed in early B cell ontogeny are identical to those encoding some NAA found in adults (Logtenberg et al., 1989; Schutte et al., 1991). In contrast, B cells that are recruited in response to foreign antigen express a diverse array of V gene segments. Any of the gene segments used for autoantibody responses may, however, be used to respond to a 'foreign' antigen.

Analysis of V genes of natural, 'foreign antigen'-induced and autoimmune disease-associated human monoclonal antibodies has delineated some of the structural features of the respective antigen-binding sites. Most NAA are directly encoded by the germline genes and are subjected to practically no mutation (Baccala et al., 1989; Sanz et al., 1989; Chen et al., 1991). In contrast, germline encoded reactivity to 'foreign antigen' is rare. By using an artificially generated 'germline revertant' (unmutated) from somatically-mutated wild-type monoclonal antibody directed against insulin, it was shown that the revertant binds the antigen in a dose-saturable and specific manner. However, the relative avidity of the revertant was more than threefold lower than that of its wild-type counterpart (Ichiyoshi et al., 1995). These results suggest that germline-encoded NAA have the potential of undergoing somatic mutation, possibly leading to the generation of mutated disease-associated autoantibodies. Several NAA and 'foreign antigen'-induced antibodies have been shown to utilize copies of the same V_H gene to encode polyreactive and monoreactive antigen-binding sites, respectively, indicating that primary structures other than those encoded by V_H and V_L genes are critical for polyreactivity. The importance of the CDR3 of the H chain in the polyreactivity of antibodies has been elegantly demonstrated by grafting an originally monoreactive IgG molecule, with an H chain CDR3 derived from a polyreactive monoclonal antibody (Schettino et al., 1996). Studies demonstrating that the presence or absence of H chain CDR3 sequence governs the acquisition or abolition of polyreactivity, indicate that the somatically generated H chain CDR3 provides the critical structure for multiple antigen binding (Ichiyoshi and Casali, 1994). The role of H chain CDR3 in polyreactivity was further defined by gene swapping and site-directed mutagenesis experiments, using two monoclonal antibodies that utilize the same $V_\kappa3$ gene, Humkv325, and V_H1 gene, 51p1, sequences in identical configuration, but having different lengths and composition of the H chain CDR3 (Crouzier et al., 1995). The L chain contributes minimally to monoreactivity and to the affinity of monoreactive monoclonal antibody (Crouzier et al., 1995).

Disease-associated autoantibodies often utilize structures for antigen recognition that are equiva-

lent to those of their naturally occurring counterparts. It has been argued that most disease-associated autoantibodies arise from naturally occurring unmutated autoantibody templates through a process of somatic diversification and self antigen-driven selection similar to that involved in the affinity maturation of 'foreign antigen'-specific antibodies. Antigenic pressure onto a polyreactive NAA-producing cell precursor B cell clone may lead to somatic mutation and the selection processes that result in not only higher affinity for antigen but also in loss of polyreactivity (Andris and Capra, 1996). A major implication is that the difference between NAA and autoantibodies emerging in patients with autoimmunity is subtle. Thus, a review of the published literature indicates that V gene usage, extent of mutations, affinity, and degree of polyreactivity do not delineate between disease-associated and natural autoantibodies. This certainly argues in favor of the existence of intrinsic mechanisms that regulate autoreactive repertoires in the healthy state of the immune system.

4. Target autoantigens and natural autoantibody repertoires

The antigenic specificity of NAA has been extensively investigated by ELISA, using immunoglobulins from serum or immunoglobulins secreted by hybridomas derived from normal B cells and panels of purified self antigens (Guilbert et al., 1982; Avrameas et al., 1983). In man and mouse, NAA were shown to bind to a broad range of evolutionarily conserved cell surface, intracellular and circulating antigens (Maire et al., 1989; Yadin et al., 1989; Pfueller et al., 1990). NAA react with self antigens that are also targets for autoantibodies in autoimmune disease, e.g. thyroglobulin (Tg), cytoplasmic antigens of polynuclear neutrophils, intrinsic factor, factor VIII (FVIII) and glomerular basement membrane (Rossi et al., 1990; Algiman et al., 1992; Dietrich et al., 1992). However, the fine epitopic specificity of NAA may differ from that of pathogenic autoantibodies as shown in the case of autoantibodies to Tg, DNA and endothelial cells (Pechaczyk et al., 1987; Bouanani et al., 1989; Bresler et al., 1990; Naito et al., 1990; Sabbaga et al., 1990; Bouanani et al., 1991; Dietrich et al., 1991; Ronda et al., 1994). Studies aimed at characterizing autoreactive repertoires using ELISA or analogous immunochemical approaches, remain limited by the small number of purified proteins available as sources of self (homologous or heterologous) antigens. More recently, a quantitative immunoblotting technique has been developed that permits the simultaneous assessment of multiple antibody reactivities with a large panel of self antigens in solubilized extracts from normal homologous tissues (Nobrega et al., 1993). This approach allows for the assessement of global repertoires of immunoreactivities toward a given tissue extract and for comparisons between the repertoires of different individuals. Using this technique, we have observed that the repertoire of reactivities of cord-blood IgM is restricted to a limited set of self proteins and is highly conserved among healthy newborns (Mouthon et al., 1995b). Similar results have been obtained upon analysis of antibody repertoires expressed early after birth in mice (Nobrega et al., 1996). The data suggest that: (i) the germline repertoire encoding IgM antibodies in the fetus, has been evolutionarily selected for reactivity with self antigens; or that (ii) the expressed neonatal B cell repertoire is selected for recognition of self during fetal development. Serum concentrations of IgM similar to those of normal adult animals are found in axenic mice, suggesting that external antigens have little if any influence on the level of NAA IgM (Pereira et al., 1986). Analysis of human IgM repertoires has shown that, to some degree, autoreactive repertoires increase in diversity during the first 2 years of life (Mouthon et al., 1996), suggesting that encountering 'foreign' antigens cross-reactive with self or exposure to novel autologous antigens (either antigens or idiotopes, e.g. those expressed by IgG) during early childhood, is responsible for further maturation of the self-reactive antibody repertoire. We also observed that the repertoire of autoreactive IgM in the serum of patients with hyper-IgM syndrome, is heterogeneous among patients and harbors a broader range of reactivities than that of healthy individuals (Lacroix-Desmazes et al., 1997), emphasizing the requirement

for T cells in the establishment of the normal autoreactive B cell repertoire. Taken together, these findings suggest that the fetal IgM self-reactive antibody repertoire is restricted to only a small extent by the genetic background, and that it is predominantly dependent on positive selection for recognition of self during ontogenesis.

We have further found that the self-reactive repertoire of IgG is established within the first 2–4 years of life and that it is highly homogeneous among children and similar to that expressed by the IgG of healthy adults (Fig. 3). Comparing the repertoires of reactivities of NAA in the serum of young infants, young adults and

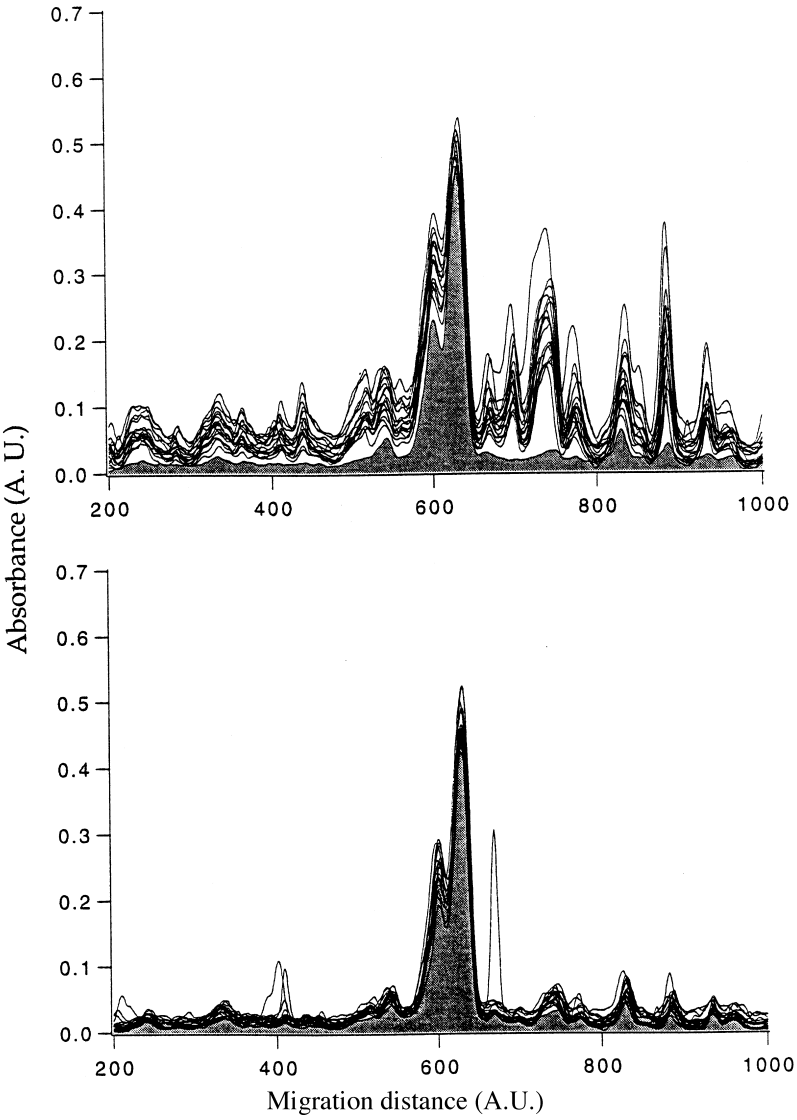

Fig. 3. Densitometric profiles of reactivity of whole serum IgG (upper panel) and of IgG purified from the serum of 18 healthy adult males (lower panel) with homologous antigens in a stomach tissue extract. IgG preparations were tested at 200 μg/ml, using a detailed procedure described in (Mouthon et al., 1995b). The reactivity profile of the IgG of each individual is shown as a full-line curve. Shaded areas depict patterns observed in the presence of the secondary anti-Fcγ antibody alone. Migration distances and absorbance are expressed as arbitrary units (A.U.).

elderly individuals aged over 70, we observed that the self-reactive repertoires of IgM and IgG of healthy individuals are conserved among individuals in each age group, and are homogeneous between age groups (Lacroix-Desmazes et al., 1995; Mouthon et al., 1995a,b, 1996). In particular, we observed that the repertoire of self-reactivities of NAA from a single healthy individual remains stable when tested after a 20-year interval (Lacroix-Desmazes and Weksler, in preparation). In contrast with self-reactivities, the repertoires of reactivities of IgG directed toward foreign antigens are found to be heterogeneous among healthy young adults and highly diverse in elderly individuals (Lacroix-Desmazes et al., 1995). Several studies have shown an increased incidence of NAA in aged individuals (Moulias et al., 1984; Manoussakis et al., 1987; Tomer and Shoenfeld, 1988). This widely accepted concept contrasts with the lack of relationship that exists between the occurrence of NAA in aged individuals and that of overt autoimmune disease (Talor and Rose, 1991). Moreover, the most prevalent and clinically severe autoimmune diseases occur in younger people rather than in the elderly. The view of increased autoreactivity with aging is now challenged by several observations of similar titers of IgG NAA among young infants, young adults and aged individuals (Gordon and Rosenthal, 1984; Mariotti et al., 1992; Hurez et al., 1993).

Taken together, the observations summarized in this section suggest that a 'complete' and mature self-reactive IgM repertoire is acquired early in life and remains conserved throughout life. Similarly, natural IgG autoreactivity is not random but highly selected for recognition of a limited set of self antigens and remains stable with aging, whereas the repertoire of IgG reactivities toward foreign antigens is diverse and dependent on the history of each individual's immune system. In other terms, natural self-reactive IgG and IgM repertoires are positively selected under physiological conditions for reactivity with a set of self antigens common to all healthy individuals (homunculus) (Lacroix-Desmazes et al., 1995; Mouthon et al., 1995a,b). The encounter with 'foreign' antigens within the first years of life may have an additional role in shaping the mature autoreactive immune system.

5. Functions of natural autoantibodies

Several functions have been proposed for NAA under physiological conditions (Table 2). NAA bind to pathogens as a consequence of polyreactivity or cross-reactivity between self and 'foreign' epitopes. The ability of NAA to serve as opsonins for pathogens (Navin et al., 1989; Michel et al., 1990), suggests the involvement of NAA in natural 'non-specific' host defense against infection (Wilson and Miles, 1964; Boyden, 1965). By binding to self constituents altered through the aging process, NAA contribute to the clearance of catabolic products from the organism (Wilson and Miles, 1964; Michael, 1969; Elson et al., 1979; Kay et al., 1983; Galili et al., 1986; Hintner et al., 1987; Lutz et al., 1987). The hypothesis that NAA serve in the clearance of metabolic waste and senescent cells was proposed by Grabar more than 20 years ago (Grabar, 1975). It has been extensively substantiated with regard to the role of natural IgG anti-band 3 autoantibodies for the clearance of senescent erythrocytes in healthy individuals (Lutz et al., 1987). It has also been suggested that NAA endowed with anti-IgG Fc specificity facilitate the clearance of soluble immune complexes from the circulation (Hay et al., 1976). Thus, by binding to constant or V regions of IgG and/or to available epitopes on antigens complexed with antibody, NAA modify the size, clearance and phlogistic potential of circulating immune complexes.

The evidence that normal circulating immunoglobulin has anti-inflammatory properties is derived from observations of the effects of the sys-

Table 2
Functions of natural autoantibodies

First line defense against infection
Clearance of aging cells
Antigen presentation to T cells
Anti-tumoral surveillance
Anti-inflammatory activity
Selection of immune repertoires and homeostasis of autoreactivity

temic administration of intravenous immunoglobulin (IVIg) in patients with inflammatory disorders. Two mechanisms prevail in these effects:

1. the ability of normal IgG (Basta et al., 1989a) and that of IgM (Miletic et al., 1996) to bind the activated cleavage fragments of complement C3b and C4b, thus preventing the deposition of these fragments and the subsequent formation of the C5b-9 membrane attack complex on the target surface following complement activation. In addition, normal human IgG was shown to increase the rate of inactivation of C3b bound to immune complexes by the complement regulatory proteins H and I (Lutz et al., 1996). The attenuation by IgG of complement-mediated damage was demonstrated in an in vivo model of complement-driven hyperacute inflammation with damage to the endothelium where IVIg prevented lethality of Forssman shock in guinea pigs (Basta et al., 1989b).
2. The selective ability of IgG to induce the production of the anti-inflammatory cytokines IL-1ra and IL-8 without the induction of Il-1β or TNFα (Dinarello and Thompson, 1991; Poutsiaka et al., 1991; Dinarello, 1994; Ruiz de Souza et al., 1995). The induction of Il-1ra may explain the rapid and dramatic anti-inflammatory effects observed with IVIg in patients with Kawasaki's disease and other inflammatory conditions, e.g. juvenile rheumatoid arthritis. IVIg was also shown to dose-dependently inhibit Il-6 production in cultures of normal human monocytes stimulated with LPS in vitro (Andersson and Andersson, 1990).

A role for NAA in immune surveillance against cancer, has been hypothesised (Wilson and Miles, 1964; Weir and Elson, 1969; Greenberg et al., 1983). Thus, the binding of NAA to cell surface antigens on malignant cells enhances or retards tumor development (Witz et al., 1984; Chow and Bennet, 1989; Cahalon et al., 1992).

There is evidence that the binding of NAA to antigens may contribute to their internalization by antigen-presenting cells, and thus modulate the processing of antigens and their subsequent presentation to T cells (Tighe et al., 1993; Kanost and McCluskey, 1994; Thornton et al., 1994). NAA clearly serve these functions on the surface of naturally autoreactive B lymphocytes. Little is known, however, about the process underlying the presentation of self antigens to autoreactive T cells. Self-peptides derived from immunoglobulin gene products may combine with self MHC and be presented by thymic B cells or other thymic antigen presenting cells (Rudensky et al., 1990; Chen et al., 1992).

Boyden (1965) and Jerne (1984) have proposed that NAA function primarily to control autoreactivity and immune homeostasis, in healthy individuals. These concepts have been reviewed elsewhere (Varela and Coutinho, 1991) and will be referred to in the section of this review dealing with connectivity of NAA. Of essential relevance for autoimmunity, is the role of NAA in participating in the selection of autoreactive B cells and in preventing the uncontrolled expansion of specific autoreactive clones, as well as the ability of NAA through V region-dependent complementary interactions to control autoreactivity in serum under physiological conditions (Coutinho, 1989). In addition, NAA may play a role in preventing the occurrence of pathological autoimmunity by binding to microbial epitopes that are similar or identical to self (Cohen and Cooke, 1986).

6. Connectivity of natural autoantibodies

NAA recognize idiotypes expressed by immunoglobulins in autologous serum, providing the humoral immune system with a high potency for establishing V region-mediated networks from early in ontogeny (Zouali and Eyquem, 1983; Lymberi et al., 1985; Holmberg et al., 1986; Muryoi et al., 1988; Dietrich et al., 1992). By interacting with surface Ig molecules, NAA may activate or anergise B lymphocytes and participate in shaping the repertoire of B cells, including the expression of their own repertoire (Jerne, 1985). The ability of normal IgG to bind to idiotypes of the T cell receptor (Marchalonis et al., 1992, 1994) provides NAA with a potential role in selecting T cell repertoires. The regulatory role of

connected NAA in immune homeostasis is evident throughout life.

Idiotypic connectivity has been demonstrated within autologous neonatal IgM in mice and in man (Holmberg et al., 1984, 1986a; Guigou et al., 1991). The infusion of minute amounts of natural IgM to newborn mice drastically reduces the expression of idiotypically-connected natural IgM later in life with long-lasting down-regulation of B cell clones expressing the target idiotypes (Lundkvist et al., 1989). The latter effects of IgM NAA are directly relevant for tolerance to self antigens in the adult life.

Murine maternal IgG is transferred through the placenta to the fetus during pregnancy and its reactivity towards self antigens is masked through V region-dependent interactions with fetal IgM (Fig. 4). Using homozygous μ-chain knock-out mice (μMT/μMT) obtained after disruption of one of the membrane exons of the gene encoding the μ-chain constant region (Kitamura et al., 1991), we have shown that maternal IgG increases the number of pre-B cells in the bone marrow and the number of B cells, both in the bone marrow and at the periphery (Malanchere et al., 1997). Maternal IgG also decreases the titer of serum IgM in newborn mice. However, there is an increase in the amount of newborn IgM capable of interacting with $F(ab')_2$ fragments of IgG (Fig. 5) (Malanchere et al., 1997). Thus, transplacental IgG not only passively protects the newborn, but selects B cell repertoires in fetal life by providing the fetus with connected antibodies (Hahn-Zoric et al., 1992). The relevance of the immunomodulating role of transferred maternal immunoglobulin to offsprings in the human situation is best illustrated by the fact that babies who have been breast-fed respond better to vaccines and exhibit fewer autoimmune diseases (Hanson and Telemo, 1997).

In adult serum, there is a large body of evidence that IgG contains antibodies capable of interacting through V regions with V regions of antibodies within the same individual's IgG fraction of serum, as demonstrated by affinity chro-

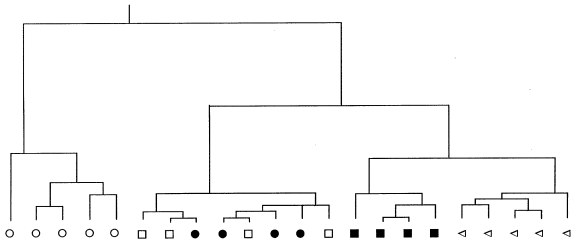

Fig. 4. Hierarchical clustering of repertoires of IgG reactivities in the serum of newborn mice with antigens in a liver tissue extract. Immunoblotting was performed using sera of 7-day-old newborn mice (△) and sera of 1-day-old newborn mice alone (○) or together with monoclonal IgM purified from a hybridoma derived from the fusion of spleens of 6-day-old naive newborn mice (BAN 1:2.91: □ and BAN 4:2.2: ■), or with polyclonal IgM purified from a pool of adult mouse serum (●). IgG was tested at 100 μg/ml and the IgM concentration was 29.4 μg/ml. Height of individual peaks of reactivity were calculated for each mouse and repertoires of peak values were subjected to hierarchical cluster analysis.

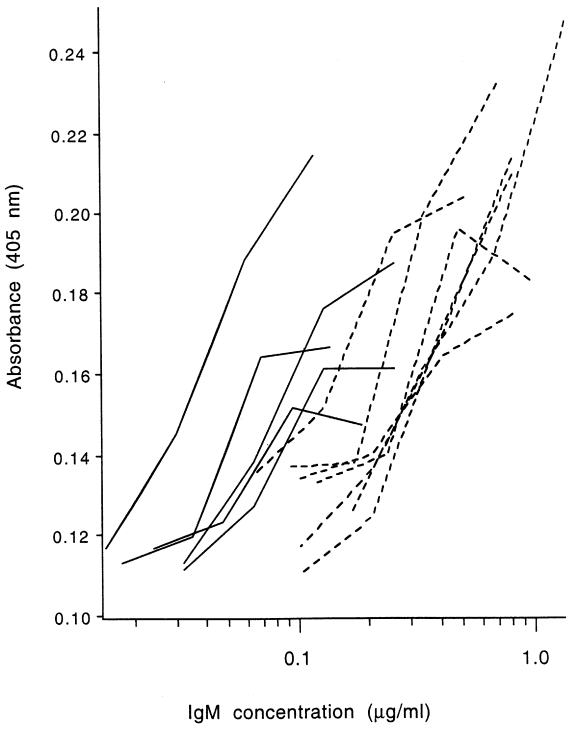

Fig. 5. Connectivity of IgM of newborn mice born from μMT/μMT (full line curves) and μMT/+ (broken line curves) mothers. Sera were diluted 1/10 to 1/80 and incubated in an ELISA plate coated with $F(ab')_2$ fragments from a polyclonal IgG preparation. Bound IgM was revealed using a specific anti-mouse IgM antibody coupled to peroxidase.

matography of $F(ab')_2$ of normal IgG on Sepharose-bound autologous IgG $F(ab')_2$ (Dietrich et al., 1992b; Vassilev et al., 1995). V region-dependent connectivity between homologous IgG molecules may be directly demonstrated using IEF of IgG $F(ab')_2$ fragments followed by immunoblotting (Fig. 6) (Ayouba et al., 1996). Whereas repertoires of reactivities towards homologous V-regions of IgG in the serum of healthy individuals exhibit homogeneous and stable patterns, repertoires of patients with systemic lupus erythematosus (SLE) differ from those of healthy individuals and change with time (Ayouba et al., 1997).

As discussed above, self-reactive antibody repertoires of natural IgG and IgM are selected for recognition of a conserved set of autoantigens and remain stable throughout life in both mouse and man (Lacroix-Desmazes et al., 1995; Mouthon et al., 1996; Nobrega et al., 1997). In whole serum however, IgG autoreactivity is 'masked' as compared with the autoreactivity of IgG purified from serum and tested under similar conditions (Chen et al., 1989; Adib et al., 1990; Saenko et al., 1992; Hurez et al., 1993; Mouthon et al., 1995). Thus, whereas the patterns of antibody reactivity of purified IgG with self antigens exhibit striking homogeneity among healthy individuals (Fig. 3), the repertoires of reactivity of IgG tested in whole serum appear to be limited to a few self antigens, and are heterogeneous among donors (Lacroix-Desmazes et al., 1995; Mouthon et al., 1995). The individual repertoires of reactivity in whole serum remain stable throughout life and have been referred to as the 'antibody immuno-finger printing' of each individual (Francoeur, 1988). The binding of purified IgG to self antigens is inhibited by the autologous non-IgG fraction of the serum in a dose-dependent fashion in vitro (Hurez et al., 1993b). Several serum factors may act simultane-

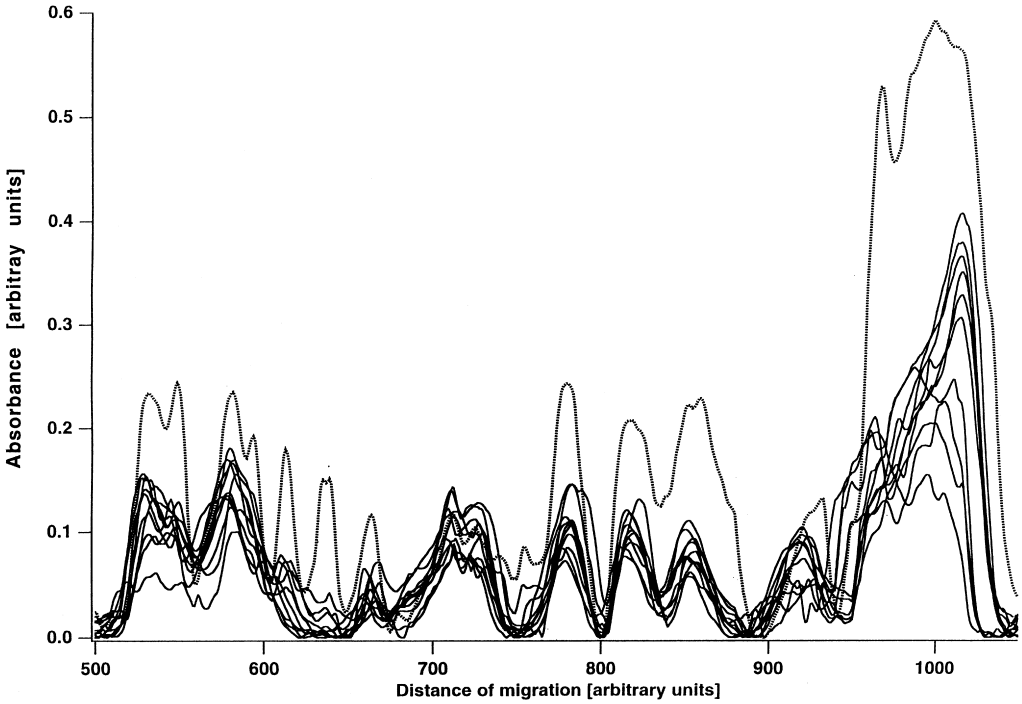

Fig. 6. Densitometric profiles of reactivity of IgG purified from the serum of 10 healthy adult donors with $F(ab')_2$ fragments from human IVIg. $F(ab')_2$ fragments from IVIg were sujected to IEF and blotted onto nitrocellulose. Biotinylated IgG preparation were tested at 100 μg/ml. The reactivity profile of the IgG from each individual is shown as a full-line curve. The migration profile of $F(ab')_2$ fragments from IVIg is shown as a dotted-line curve. Migration distances and absorbance are expressed as arbitrary units.

ously to control IgG autoreactivity in unfractionated serum (Saenko et al., 1992): (i) IgG in whole serum interacts with multiple circulating antigens, masking autoreactivity to some degree, unless immune complexes are dissociated by heating or by repeated freezing and thawing (personal observation); (ii) IgG in serum interacts with autologous IgM. Thus, purified IgM inhibits the binding of autologous IgG to self antigens by competing with IgG for binding to the antigen and by direct idiotypic (V region-complementary) interactions with natural IgG autoantibodies (Adib et al., 1990; Hurez et al., 1993). Purified human IgM binds to $F(ab')_2$ fragments of autologous IgG and soluble $F(ab')_2$ fragments of IgG inhibit this interaction (Hurez et al., 1993a).

In addition to IgM–IgM interactions and to IgM–IgG interactions, V regions of IgG interact with each other within the IgG fraction of the serum of healthy individuals to control IgG reactivity (Tankersley et al., 1988; Roux and Tankersley, 1990; Dietrich et al., 1992; Vassilev et al., 1995). These interactions between connected IgG molecules only become apparent after immunoaffinity purification or other techniques that would dissociate idiotype-anti-idiotype complexes of IgG.

The relevance of these peripheral mechanisms of control of IgG autoreactivity is emphasized by the observations that remission in various autoimmune diseases may be associated with increased connectivity whereas acute flares of autoimmunity may be associated with the lack of detectable peripheral control. Thus, remission or recovery from autoimmune diseases such as myasthenia gravis, the Guillain–Barré syndrome, systemic vasculitis with anti-neutrophil cytoplasmic antigen autoantibodies, SLE, anti-FVIII autoimmune disease, anti-fibrinogen autoimmune disease have been shown to be associated with the presence in autologous serum of 'protective' anti-idiotypic antibodies that neutralize the activity of pathogenic autoantibodies of the patients (Dwyer et al., 1983; Zouali and Eyquem, 1983; Sultan et al., 1987; Ruiz-Arguelles, 1988; van Doorn et al., 1990; Rossi et al., 1991). Such neutralizing antibodies contributing to peripheral control of autoreactivity are also found in healthy individuals under normal conditions, as exemplified in the case of anti-FVIII NAA (Algiman et al., 1992; Gilles and Saint-Remy, 1994). During the acute phase of an autoimmune disease, the specific activity of disease-associated autoantibodies, e.g. anti-endothelial cell antibodies (AECA) in IgG of patients with SLE, has been found to be identical in the purified IgG fraction of serum and in whole serum, suggesting that the serum factors involved in peripheral control of autoreactivity are inefficient or absent (Hurez et al., 1993b; Ronda et al., 1994). These considerations are directly relevant for the inhibitory activity of intravenous immunoglobulin in patients with autoimmune diseases, as demonstrated originally for the idiotypic neutralization of autoantibodies to Factor VIII in vitro and in vivo in patients with anti-Factor VIIII autoimmune disease (Sultan et al., 1987; Kazatchkine et al., 1994). In contrast with autoantibodies, antibodies directed against vaccinal non-self antigens (e.g. tetanus toxoid) appear not to be controlled in whole serum (Hurez et al., 1993b), indicating that the control of IgG reactivity dependent on the connectivity of NAA is primarily restricted to the control of autoreactive clones.

7. Significance of NAA: the boundaries between NAA and pathogenic autoantibodies

Autoreactivity in healthy individuals has been recognized for many years. Besredka (1901) reported the presence of antibodies neutralizing the hemolytic action of anti-erythrocyte autoantibodies in the serum of normal animals in 1901 and Landsteiner (1945) described the presence of antibodies reacting with self antigens in the sera of healthy individuals 50 years ago. The paradigm that autoreactivity is necessarily associated with disease has, however, prevailed among immunologists since the formulation of the clonal selection theory and the early observations of autoantibodies in patients with the first identified autoimmune diseases in the early 1960s (Burnet, 1959). It was not before Jerne that it became accepted that autoreactivity, i.e. reactivity of antibodies with variable regions of autologous immunoglobulins, exists in the absence of autoim-

mune manifestations, and is an essential element of the normal immune system (Jerne, 1974). Based on molecular similarities between immunoglobulin molecules and adhesion molecules, in particular the use of a common and evolutionarily conserved immunoglobulin domain, a recent hypothesis has even proposed that natural antibodies have primarily evolved for recognizing self, the ability of antibodies to recognise non-self having been acquired later in evolution (Stewart, 1992). In this respect, the immune system appears indeed as an 'extraordinary tool for self assessment' (Avrameas et al., 1981).

The boundaries between physiological autoreactivity and pathological autoimmunity remain ill-defined. Pathogenic autoantibodies have been claimed to be primarily of the IgG isotype, encoded by highly mutated V genes, to exhibit high affinity binding to self antigens and to be oligoreactive (Andris and Capra, 1996). There are, however, only few conditions in experimental animals (e. g., spontaneous lupus-like disease in MLR *lpr/lpr* mice (Hahn, 1981) and man) (Lindstrom et al., 1988) where antibodies eluted from damaged tissues and from patients' sera have been shown to exhibit such characteristics. Evidence for autoantigen-driven expansion of self-reactive clones is lacking in most autoimmune disorders and there is evidence in several autoimmune conditions, that disease-associated autoantibodies exhibit characteristics similar to those of NAA. This is, for example, the case with antibodies expressing the 16.6 idiotype, the serum titer of which correlates with disease activity in patients with SLE (Isenberg et al., 1984). In addition, under certain conditions, NAA may be induced to express pathogenic potential, as shown by the induction of experimental SLE by immunization of naive mice with the 16.6 antibody (Mendlovic et al., 1988) or induction of the anti-phospholipid syndrome with anti-cardiolipin NAA (Bakimer et al., 1992).

Taken together, the experimental and clinical observations and concepts that have accumulated in the last 20 years suggest two different pathways of induction of autoimmune disease: (i) in certain conditions, the pathogenic autoantibodies would indeed represent an oligoclonal immune response to a self antigen that has undergone physico–chemical alterations or is abnormally presented to autoreactive autologous T cells, generating autoantibodies that exhibit most characteristics of 'immune' antibodies; (ii) alternatively, autoimmune diseases would arise from a primary (e.g. genetic) or secondary (e.g. infectious) alteration in the structure or function of the immune network that ensures homeostasis of autoreactivity under physiological conditions, resulting in the uncontrolled emergence and expansion of self-reactive clones that may exhibit characteristics of NAA or of immune antibodies. Discriminating between these alternatives has obvious implications for therapeutic strategies in autoimmune disorders.

8. A therapeutic role for natural autoantibodies

Intravenous immunoglobulin (IVIg) is a therapeutic preparation of pooled normal polyspecific human IgG obtained from large numbers of healthy donors. IVIg thus contains the wide spectrum of NAA and 'immune' antibodies expressed in normal human serum. IVIg was originally used as substitutive therapy for the treatment of pri-

Table 3
Experimental autoimmune diseases that are prevented or attenuated by IVIg or homologous NAA

Disease	Reference
$HgCl_2$-induced vasculitis and glomerular disease	Rossi et al. (1991a)
Lupus-like disease of (NZB × NZW)F1 and of *lpr/lpr* mice	Sundblad et al. (1994)
Experimental autoimmune uveitis	Saoudi et al. (1993)
Atherosclerosis in ApoE knock-out mice	Nicoletti et al. (1998)
Experimental allergic encephalomyelitis	Pashov et al. (1996)
Experimental encephalomyelitis induced by Theiler's virus	Miller and Rodriguez (1995)
Diabetes in NOD-mice	Andersson et al. (1991)

mary and secondary antibody deficiencies. The realisation of a beneficial effect of IVIg in patients with autoimmune thrombocytopenic purpura (Imbach et al., 1981) and other autoimmune diseases involving pathogenic autoantibodies or autoreactive T cells, has now lead to its use in a broad range of autoimmune and systemic inflammatory disorders (Kazatchkine et al., 1994; Dalakas, 1997). IVIg, normal homologous immunoglobulins and natural monoclonal antibodies have also been shown to exert immunoregulatory potential in experimental models of autoimmune diseases (Table 3).

Several mutually non-exclusive mechanisms underlying the beneficial effects of IVIg in autoimmune diseases have been documented (Kazatchkine and Kaveri, 1997). The effects of IVIg involve both the variable regions and the Fc portion of the IgG molecule. Fc/Fc receptor-mediated effects include the functional blockade of Fc receptors on phagocytes and secondary cellular responses (e.g. of B cells) induced by triggering of Fcγ receptors. Blockade of Fc receptors accounts, to a large extent, for the beneficial effect of IVIg in peripheral autoimmune thrombocytopenias. Constant regions of IgG are also involved in the anti-inflammatory properties of IVIg, including attenuation of complement-mediated tissue damage and induction of anti-inflammatory cytokines (see above and Ruiz de Souza et al., 1995). The other immunoregulatory properties of IVIg are primarily dependent on the V regions of infused antibodies, although most of these effects require cooperative interactions between Fc fragments and Fc receptors on cells that are targeted by the relevant variable regions of IVIg (Xu et al., 1997). Variable region-dependent effects of IVIg include: (i) the neutralization of pathogenic antibodies by anti-idiotypes present in IVIg, a mechanism that is responsible for the early decrease in the titer of circulating autoantibodies in patients with anti-FVIII autoimmune disease and ANCA-positive vasculitis following infusion of IVIg (Sultan et al., 1984; Jayne et al., 1993); (ii) neutralization of superantigens which results in inhibition of superantigen-elicited T cell activation; (iii) the long-term control of the expansion and activation of lymphocyte subsets, and selection of immune repertoires. Evidence in experimental animals and in man indicates that normal immunoglobulin selects pre-immune B cell repertoires in the bone-marrow and peripheral lymphoid tissues (Sundblad et al., 1991; Dietrich et al., 1993); infusion of IVIg or homologous NAA to autoimmune animals or individuals restores patterns of fluctuations of serum autoantibody levels similar to those seen in healthy controls (Dietrich et al., 1993); in man, prolonged suppression of disease-related autoantibodies has been observed in patients with autoimmune diseases

Table 4
Examples of self antigens recognized by NAA in IVIg

Idiotypes/variable regions of antibodies and surface Ig on B cells	Rossi and Kazatchkine (1989)
Idiotype, framework and constant regions of T-cell receptors	Rossi et al. (1989), Kaveri et al. (1990), Marchalonis et al. (1992)
CD4	Hurez et al. (1994)
CD5	Vassilev et al. (1993)
MHC class I molecules	Kaveri et al. (1996)
RGD sequence on ligands of adhesion molecules	Vassilev et al. (1997)
Fcγ receptors	Martinvalet et al. (in preparation)
Autologous blood group antigens	Spalter et al. (1997)
FVIII	Algiman et al. (1992)

treated with IVIg, far beyond the half-life of infused immunoglobulins (Sultan et al., 1984); in addition, an increased concentration of serum IgM and the selective up-regulation or down-regulation of specific B cell clones expressing complementary idiotypes to variable regions of antibodies in IVIg, follow the infusion of IVIg in patients with autoimmune diseases (Dietrich et al., 1993). There are a number of interactions of IVIg with target molecules and cells that may account for the complex events that underly the selection of repertoires by IgG. Examples of molecules that are critical for immuno-regulation and that are recognized by specific NAA in therapeutic IVIg preparations are listed in Table 4.

We believe that the immunomodulatory effects of IVIg are dependent on the content in NAA of IVIg preparations. Further analysis of the mechanisms of action of IVIg in autoimmune diseases should shed more light on the functions of NAA.

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References

Adib-Conquy, M., Avrameas, S., Terninck, T., 1993. Monoclonal IgM and IgG autoantibodies obtained after polyclonal activation, show reactivities similar to those of polyclonal natural autoantibodies. Molec. Immunol. 30, 119.

Adib, M., Ragimbeau, J., Avrameas, S., Ternynck, T., 1990. IgG autoantibody activity in normal mouse serum is controlled by IgM. J. Immunol. 145, 3807.

Algiman, M., Dietrich, G., Nydegger, U., Boieldieu, D., Sultan, Y., Kazatchkine, M.D., 1992. Natural antibodies to factor VIII (anti-hemophilic factor) in healthy individuals. Proc. Natl. Acad. Sci. U.S.A. 89, 3795.

Alt, F., Blackwell, T., Yancopoulos, G., 1987. Development of the primary antibody repertoire. Science 238, 1079.

Andersson, J.P., Andersson, U.G., 1990. Human intravenous immunoglobulin modulates monokine production in vitro. Immunology 71, 372.

Andersson, A., Forsgren, S., Soderstrom, A., Holmberg, D., 1991. Monoclonal natural antibodies prevent development of diabetes in the non-obese diabetic (NOD) mouse. J. Autoimmun. 4, 733.

Andris, J.S., Capra, J.D., 1996. Variable region gene utilization in human antibodies to exogeneous antigens. In: Zanetti, M., Capra, J.D. (Eds.), The Antibodies. Harwood Academic Publishers, Amsterdam, p. 1.

Antin, J.H., Emerson, S.G., Martin, P., Gadol, N., Ault, A., 1986. Leu1 + ($CD5^+$) B cells: a major lymphoid subpopulation in human fetal spleen: phenotypic and functional studies. J. Immunol. 136, 505.

Araga, S., Kishimoto, M., Nakayasu, A.A.H., Takenaka, T., Takahshi, a.K., 1995. The $CD5^+$ cells and myasthenia gravis. Autoimmunity 20, 129.

Avrameas, S., 1991. Natural autoantibodies: from 'horror autotoxicus' to 'gnothi seauton'. Immunol. Today 12, 154.

Avrameas, S., Ternynck, T., 1995. Natural autoantibodies: the other side of the immune system. Res. Immunol. 146, 235.

Avrameas, S., Guilbert, B., Dighiero, G., 1981. 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. Inst. Pasteur Immunol. 132C, 231.

Avrameas, S., Dighiero, G., Lymberi, P., Guilbert, B., 1983. Studies on natural antibodies and autoantibodies. Ann. Inst. Pasteur Immunol. 134D, 103.

Ayouba, A., Peltre, G., Coutinho, A., 1996. Quantitative analysis of multiple V region interactions among normal human IgG. Eur. J. Immunol. 26, 710.

Ayouba, A., Ferreira, C., Coutinho, A., 1997. Distinguishable patterns of connectivity in serum immunoglobulins from SLE patients and healthy individuals. Scand. J. Immunol. 45, 408.

Baccala, R., Vo Quang, T., Gilbert, M., Ternynck, T., Avrameas, S., 1989. Two murine natural polyreactive autoantibodies are encoded by nonmutated germ-line genes. Proc. Natl. Acad. Sci. U.S.A. 86, 4624.

Bakimer, R., Fishman, P., Blank, M., Sredni, B., Djaldetti, M., Shoenfeld, Y., 1992. Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anticardiolipin antibody (H-3). J. Clin. Invest. 89, 1558.

Basta, M., Langlois, P.F., Marques, M., Frank, M.M., Fries, L.F., 1989a. High-dose intravenous immunoglobulin modifies complement-mediated in vivo clearance. Blood 74, 326.

Basta, M., Kirshbom, P., Frank, M.M., Fries, L.F., 1989b. Mechanism of therapeutic effect of high-dose intravenous immunoglobulin. Attenuation of acute, complement-dependent immune damage in a guinea pig model. J. Clin. Invest. 84, 1974.

Bendtzen, K., Svenson, M., Jonsson, V., Hippe, E., 1990.

Autoantibodies to cytokines — friends or foes? Immunol. Today 11, 167.

Berman, J.E., Nickerson, K.G., Pollock, R.R., et al., 1991. VH gene usage in humans: biased usage of the VH6 gene in immature B lymphoid cells. Eur. J. Immunol. 21, 1311.

Besredka, M., 1901. Les antihémolysines naturelles. Ann. Inst. Pasteur 15, 758.

Bikah, G., Carey, J., Ciallella, J.R., Tarakhovsky, A., Bondada, S., 1996. CD5-mediated negative regulation of antigen receptor-induced growth signals in B-1 B cells. Science 274, 1906.

Bouanani, M., Piechaczyk, M., Pau, B., Bastide, M., 1989. Significance of the recognition of certain antigenic regions on the thyroglobulin molecule by natural autoantibodies from healthy subjects. J. Immunol. 143, 1129.

Bouanani, M., Bataille, R., Piechaczyk, M., Salhi, S.L., Pau, B., Bastide, M., 1991. Respective epitopic specificity patterns of anti-human thyroglobulin autoantibodies in patients with Sjrögren's syndrome and patients with Hashimoto's thyroiditis. Arthritis Rheum. 34, 1585.

Boyden, S., 1965. Natural antibodies and the immune response. Adv. Immunol. 5, 1.

Bresler, H.S., Burek, L.C., Hoffman, W.H., Rose, N.R., 1990. Autoantigenic determinants on human thyroglobulin: determinants recognized by autoantibodies from patients with chronic autoimmune thyroiditis compared to auto-antibodies from healthy subjects. Clin. Immunol. Immunopathol. 54, 76.

Burastero, S.E., Casali, P., Wilder, R.L., Notkins, A.L., 1988. Monoreactive high affinity and polyreactive low affinity rheumatoid factors are produced by $CD5^+$ B cells from patients with rheumatoid arthritis. J. Exp. Med. 168, 1979.

Burnet, M., 1959. The Clonal Selection Theory of Acquired Immunity. Vanderbilt University Press, Nashville.

Cahalon, L., Korem, S., Gonen, B., Puri, J., Smorodinsky, N.I., Witz, I.P., 1992. Autoantibody-mediated regulation of tumor growth. Ann. N.Y. Acad. Sci. 651, 393.

Casali, P., Notkins, A.L., 1989a. $CD5^+$ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. Immunol. Today 10, 364.

Casali, P., Notkins, A.L., 1989b. Probing the human B-cell repertoire with EBV: polyreactive antibodies and $CD5^+$ B lymphocytes. Ann. Rev. Immunol. 7, 513.

Chen, P.P., Liu, M.D., Glass, C.A., Sinha, S., Kipps, T.J., Carson, D.A., 1989. Characterization of two Ig VH genes which are homologous to human rheumatoid factors. Arthritis Rheum. 32, 72.

Chen, C., Stenzel-Poore, M.P., Rittenberg, M.B., 1991. Natural auto- and polyreactive antibodies differing from antigen-induced antibodies in the H chain CDR3. J. Immunol. 147, 2359.

Chen, J.J., Kaveri, S., Kohler, H., 1992. Cryptic T cell epitopes in polymorphic immunoglobulin regions: evidence for positive repertoire selection during fetal development. Eur. J. Immunol. 22, 3077.

Chen, Z.J., Wheeler, J., Notkins, A.L., 1995. Antigen-binding B cells and polyreactive antibodies. Eur. J. Immunol. 25, 579.

Chow, D., Bennet, R., 1989. Low natural antibody and low in vivo tumor resistance, in xid-bearing B-cell deficient mice. J. Immunol. 142, 3702.

Cohen, I.R., Cooke, A., 1986. Natural autoantibodies might prevent autoimmune disease. Immunol. Today 7, 363.

Coutinho, A., 1989. Beyond clonal selection and network. Immunol. Rev. 110, 63.

Coutinho, A., Kazatchkine, M.D., 1994. Autoimmunity. Physiology and Disease. John Wiley, New York.

Coutinho, A., Kazatchkine, M.D., Avrameas, S., 1995. Natural autoantibodies. Curr. Opin. Immunol. 7, 812.

Crouzier, R., Martin, T., Pasquali, J.L., 1995. Heavy chain variable region, light chain variable region, and heavy chain CDR3 influences on the mono- and polyreactivity and on the affinity of human monoclonal rheumatoid factors. J. Immunol. 154, 4526.

Cuisinier, A.-M., Guigou, V., Boubli, L., Fougereau, M., Tonnelle, C., 1989. Preferential expression of Vh5 and Vh6 immunoglobulin genes in early human B-cell ontogeny. Scand. J. Immunol. 30, 493.

Dalakas, M.C., 1997. Intravenous immune globulin therapy for neurologic diseases. Ann. Intern. Med. 126, 721.

Diaw, L., Magnac, C., Pritsch, O., Buckle, M., Alzari, P.M., Dighiero, G., 1997. Structural and affinity studies of IgM polyreactive natural antibodies. J. Immunol. 158, 968.

Dietrich, G., Piechaczyk, M., Pau, B., Kazatchkine, M.D., 1991. Evidence for a restricted idiotypic and epitopic specificity of anti-thyroglobulin autoantibodies in patients with autoimmune thyroiditis. Eur. J. Immunol. 21, 811.

Dietrich, G., Algiman, M., Sultan, Y., Nydegger, U.E., Kazatchkine, M.D., 1992a. Origin of anti-idiotypic activity against anti-factor VIII autoantibodies in pools of normal human immunoglobulin G (IVIg). Blood 79, 2946.

Dietrich, G., Kaveri, S.V., Kazatchkine, M.D., 1992b. A V region-connected autoreactive subfraction of normal human serum immunoglobulin G. Eur. J. Immunol. 22, 1701.

Dietrich, G., Varela, F., Hurez, V., Bouanani, M., Kazatchkine, M.D., 1993. Selection of the expressed B cell repertoire by infusion of normal immunoglobulin G in a patient with autoimmune thyroiditis. Eur. J. Immunol. 23, 2945.

Dighiero, G., Lymberi, P., Mazie, J.-C., Rouyre, S., Butler-Browne, G.S., Avrameas, S., 1983. Murine hybridomas secreting natural monoclonal antibodies reacting with self antigens. J. Immunol. 131, 2267.

Dighiero, G., Poncet, P., Rouyre, S., Mazie, J., 1986. Newborn Xid mice carry the genetic information for the production of natural autoantibodies against DNA, cytoskeletal proteins, and TNP. J. Immunol. 136, 4000.

Dinarello, C.A., 1994. Is there a role for Interleukin-1 blokade in Intravenous Immunoglobulin therapy. Immunol. Rev. 139, 173.

Dinarello, C., Thompson, R.C., 1991. Blocking IL-1: interleukin 1 receptor antagonist in vivo and in vitro. Immunol. Today 12, 404.

Durandy, A., Thuillier, L., Forveille, M., Fischer, A., 1990.

Phenotypic and functional characteristics of human newborns' B lymphocytes. J. Immunol. 144, 60.

Durandy, A., Schiff, C., Bonnefoy, J.Y., et al., 1993. Induction by anti-CD40 ligand and cytokines of IgG, IgA and IgE production by B cells from patients with X-linked IgM syndrome. Eur. J. Immunol. 23, 2294.

Dwyer, D.S., Bradley, R.J., Urquhart, C.K., Kearney, J.F., 1983. Naturally occurring anti-idiotypic antibodies in myasthenia gravis patients. Nature 301, 611.

Elson, C.J., Williams, N.A., 1995. Autoreactive T cells repertoire. Immunol. Today 16, 71.

Elson, C.J., Naysmith, J.D., Taylor, R.B., 1979. B-cell tolerance and autoimmunity. Int. Rev. Exp. Pathol. 19, 137.

Filion, M.C., Bradley, A.J., Devine, D.V., Décary, F., Chartrand, P., 1995. Autoreactive T cells in healthy individuals show tolerance in vitro with characteristics similar to but distinct from clonal anergy. Eur. J. Immunol. 25, 3123.

Francoeur, A.M., 1988. Antibody fingerprinting: a novel method for identifying individual people and animals. Biotechnology 6, 822.

Freitas, A.A., Viale, A.C., Sundblad, A., Heusser, C., Coutinho, A., 1991. Normal serum immunoglobulins participate in the selection of peripheral B-cell repertoires. Proc. Natl. Acad. Sci. U.S.A. 88, 5640.

Galili, U., Clark, M.R., Shohet, S.B., 1986. Excessive binding of natural anti-alpha-galactosyl immunoglobulin G to sickle erythrocytes may contribute to extra-vascular cell destruction. J. Clin. Invest. 77, 27.

Galili, U., Basbaum, C.B., Shohet, S.B., Buehler, J., Macher, B.A., 1987. Identification of erythrocyte Gala1-3Gal glycosphingolipids with a mouse monoclonal antibody, Gal-13. J. Biol. Chem. 262, 4683.

Gilles, J.G., Saint-Remy, J.M., 1994. Healthy subjects produce both anti-factor VIII and specific anti-idiotypic antibodies. J. Clin. Invest. 94, 1496.

Gordon, J., Rosenthal, M., 1984. Failure to detect age-related increase of non-pathological autoantibodies. Lancet i, 231.

Grabar, P., 1975. Hypothesis. Autoantibodies and immunological theories: an analytical review. Clin. Immunol. Immunopathol. 4, 453.

Greenberg, A.H., Chow, D.A., Wolosin, L.B., 1983. Natural antibodies: origin, genetics, specificity and role in host resistance to tumors. Clin. Immunol. Allergy 3, 389.

Guigou, V., Guilbert, B., Moinier, D., et al., 1991. Ig repertoire of human polyspecific antibodies and B-cell ontogeny. J. Immunol. 146, 1368.

Guilbert, B., Dighiero, G., Avrameas, S., 1982. Naturally occuring antibodies against nine common antigens in normal human sera. I. Detection, isolation, and characterization. J. Immunol. 128, 2779.

Hahn, B.H., 1981. Characteristics of pathogenic subpopulations of antibodies to DNA. Arthritis Rheum. 25, 747.

Hahn-Zoric, B., Carlsson, J., Björkander, A.D., Osterhausl, M.E., Mellander, L., Hanson, L.A., 1992. Presence of non-maternal antibodies in newborns of mothers with antibody deficiencies. Pediatric. Res. 32, 150.

Hanson, L.A., Telemo, E., 1997. Immunobiology and epidemiology of breast feeding in relation to prevention of inffections from a global perspective. In: (Eds.), Mucosal Immunol. Academic Press, p.

Hardy, R.R., Hayakawa, K., Shimizu, M., Yamasaki, K., Kishimoto, T., 1987. Rheumatoid factor secretion from human Leu-1 + B cells. Science 236, 81.

Hay, F.C., Nineham, L.J., Torrigiani, G., Roitt, I.M., 1976. 'Hidden' IgG antiglobulins in normal human serum. Clin. Exp. Immunol. 25, 185.

Hintner, H., Romani, N., Stanzl, U., Grubauer, G., Fritsch, P., Lawley, T.J., 1987. Phagocytosis of keratin filament aggregates following opsonization with IG anti-keratin filament autoantibodies. J. Invest. Dermatol. 88, 176.

Holmberg, D., Forsgren, S., Ivars, F., Coutinho, A., 1984. Reactions amongst IgM antibodies derived from normal, neonatal mice. Eur. J. Immunol. 14, 435.

Holmberg, D., Wennerström, G., Andrade, L., Coutinho, A., 1986a. The high idiotypic connectivity of 'natural' newborn antibodies is not found in adult mitogen-reactive B cell repertoires. Eur. J. Immunol. 16, 82.

Holmberg, D., Freitas, A.A., Portnoi, D., Jacquemart, F., Avrameas, S., Coutinho, A., 1986b. Antibody repertoires of normal BALB/c mice: B lymphocyte populations defined by state of activation. Immunol. Rev. 93, 147.

Huetz, F., Larsson-Sciard, E.L., Pereira, P., Portnoï, D., Coutinho, A., 1988. T cell dependence of the natural autoreactive B cell activation in the spleen of normal mice. Eur. J. Immunol. 18, 1615.

Hurez, V., Dietrich, G., Kaveri, S.V., Kazatchkine, M.D., 1993a. Polyreactivity is a property of natural and disease-associated human autoantibodies. Scand. J. Immunol. 38, 190.

Hurez, V., Kaveri, S.V., Kazatchkine, M.D., 1993b. Expression and control of the natural autoreactive IgG repertoire in normal human serum. Eur. J. Immunol. 23, 783.

Hurez, V., Kaveri, S.V., Mouhoub, A., Dietrich, G., Mani, J.C., Klatzmann, D., Kazatchkine, M.D., 1994. Anti-CD4 activity of normal human immunoglobulins G for therapeutic use (Intravenous immunoglobulin IVIg). Ther. Immunol. 1, 269.

Ichiyoshi, Y., Casali, P., 1994. Analysis of the structural correlates for antibody polyreactivity by multiple reassortments of chimeric human immunoglobulin heavy and light chain V segments. J. Exp. Med. 180, 885.

Ichiyoshi, Y., Zhou, M., Casali, P., 1995. A human anti-insulin IgG autoantibody apparently arises through clonal selection from an insulin-specific 'germ-line' natural antibody template. Analysis by V gene segment reassortment and site-directed mutagenesis. J. Immunol. 154, 226.

Imbach, P., Barandun, S., d'Apuzzo, V., et al., 1981. High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. Lancet i, 1228.

Isenberg, D.A., Shoenfeld, Y., Madaio, M.P., et al., 1984. Anti-DNA antibody idiotypes in systemic lupus erythematosus. Lancet i, 417.

Jayne, D.R.W., Esnault, V.L.M., Lockwood, C.M., 1993. ANCA anti-idiotype antibodies and the treatment of systemic vas-

culitis with intravenous immunoglobulin. J. Autoimmun. 6, 207.

Jeong, H.D., Teale, J.M., 1988. Comparison of the fetal and adult functional repertoires by analysis of VH family expression. J. Exp. Med. 168, 589.

Jeong, H.D., Teale, J.M., 1989. VH gene repertoire of resting B cells. Preferential use of D-proximal families early in development may be due to distinct B cell subsets. J. Immunol. 143, 2752.

Jerne, N.K., 1974. Towards a network theory of the immune system. Ann. Inst. Pasteur Immunol. 125C, 373.

Jerne, N.K., 1984. Idiotypic networks and other preconceived ideas. Immunol. Rev. 79, 5.

Jerne, N.K., 1985. The generative grammar of immune system. EMBO J. 4, 847.

Kanost, D., McCluskey, ?, 1994. Anergic B cells constitutively present self antigen: enhanced immunoglobulin receptor-mediated presentation of antigenic determinants by B cells is hierarchical. Eur. J. Immunol. 24, 1186.

Kasaian, M.T., Ikematsu, H., Casali, P., 1992. Identification and analysis of a novel human surface CD5- B lymphocyte subset producing natural antibodies. J. Immunol. 148, 2690.

Kaveri, S.V., Kang, C.Y., Kohler, H., 1990. Natural mouse and human antibodies bind to a peptide derived from a germline variable heavy chain: evidence for evolutionary conserved self-binding locus. J. Immunol. 145, 4207.

Kaveri, S., Vassilev, T., Hurez, V., et al., 1996. Antibodies to a conserved region of HLA class I molecules, capable of modulating CD8 T cell-mediated function, are present in pooled normal immunoglobulin for therapeutic use (IVIg). J. Clin. Invest. 97, 865.

Kay, M.M.B., Goodman, S., Whitfield, C., Wong, P., Zaki, L., Rudoloff, V., 1983. The senescent cell antigen is immunologically related to band 3. Proc. Natl. Acad. Sci. U.S.A. 80, 1631.

Kazatchkine, M.D., Kaveri, S.V. (Eds.), 1997. Therapeutic Immunomodulation with Normal Polyspecific Immunoglobulin G (Intravenous Immunoglobulin, IVIg). Harwood Academic Publishers, Langhorne.

Kazatchkine, M.D., Dietrich, G., Hurez, V., et al., 1994. V Region-mediated selection of autoreactive repertoires by intravenous immunoglobulin (IVIg). Immunol. Rev. 139, 79.

Kearney, J., Vakil, M., 1986. Idiotype-directed interactions during ontogeny play a major role in the establishment of the adult B cell repertoire. Immunol. Rev. 94, 39.

Kellerman, S.A., McCormick, D.J., Freeman, S.L., Morris, J.C., Conti-Fine, B.M., 1995. TSH receptor sequences recognized by $CD4^+$ T cells in Graves disease patients and healthy controls. J. Autoimmun. 8, 685.

Kipps, T.J., 1989. The CD5 B Cell. Adv. Immunol. 47, 117.

Kitamura, D., Roes, J., Kuhn, R., Rajewsky, K., 1991. A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. Nature 350, 423.

Kiyoi, H., Naito, K., Ohno, R., Naoe, T., 1995. Comparable profiles of the immunoglobulin heavy chain complementarity determining region (CDR)-3 in $CD5^+$ and $CD5^-$ human cord blood B lymphocytes. Immunology 85, 236.

Kohler, H., Kieber-Emmons, T., Srinivasan, S., et al., 1988. Revised immune network concepts. Clin. Immunol. Immunopathol. 52, 104.

Lacroix-Desmazes, S., Mouthon, L., Coutinho, A., Kazatchkine, M.D., 1995. Analysis of the natural human IgG antibody repertoire: life-long stability of reactivities towards self antigens contrasts with age-dependent diversification of reactivities against bacterial antigens. Eur. J. Immunol. 25, 2598.

Lacroix-Desmazes, S., Mouthon, L., Pashov, A. et al. Analysis of antibody reactivities toward self antigens of IgM of patients with Waldenström's Macroglobulinemia. Intern. Immunol., in press.

Landsteiner, K., 1945. The nature and specificity of antibodies. In: (Eds.), The Specificity of Serological Reactions. Harvard Univesity Press, Cambridge, MA, p. 127.

Lindstrom, J., Shelton, D., Fujii, Y., 1988. Myasthenia gravis. Adv. Immunol. 42, 233.

Logtenberg, T., Young, F.M., Van Es, J.H., Gmelig-Meyling, F.H.J., Alt, F.W., 1989. Autoantibodies encoded by the most Jh-proximal human immunoglobulin heavy chain variable region gene. J. Exp. Med. 170, 1347.

Lundkvist, I., Coutinho, A., Varela, F., Holmberg, D., 1989. Evidence for a functional idiotypic network among natural antibodies in normal mice. Proc. Natl. Acad. Sci. U.S.A. 86, 5074.

Lutz, H., Wipf, G., 1982. Naturally occuring autoantibodies to skeletal proteins from human red blood cells. J. Immunol. 128, 1695.

Lutz, H., Bussolino, F., Flepp, R., et al., 1987. Naturally occuring anti-band 3 antibodies and complement together mediate phagocytosis of oxidatively stressed human erythrocytes. Proc. Natl. Acad. Sci. U.S.A. 84, 7368.

Lutz, H.U., Stammler, P., Jelezarova, E., Nater, M., Spath, P.J., 1996. High doses of immunoglobulin G attenuate immune aggregate-mediated complement activation by enhancing physiologic cleavage of C3b in C3bn-IgG complexes. Blood 88, 184.

Lymberi, P., Blancher, A., Calvas, P., Avrameas, S., 1989. Natural autoantibodies in nude and normal outbred (Swiss) and inbred (BALB/c) mice. J. Autoimmun. 2, 283.

Lymberi, P., Dighiero, G., Ternynck, T., Avrameas, S., 1985. A high incidence of cross-reactive idiotypes among murine natural autoantibodies. Eur. J. Immunol. 15, 702.

Maire, M.A., Mittey, M., Lambert, P.H., 1989. The presence of cryoprecipitable immunoglobulins in normal human sera may reflect specific molecular interactions. Autoimmunity 2, 155.

Malanchere, E., Huetz, F., Coutinho, A., 1997. Maternal IgG stimulates B lineage cell development in the progeny. Eur. J. Immunol. 27, 788.

Maloum, K., Davi, F., Magnac, C., et al., 1995. Analysis of VH gene expression in $CD5^+$ and $CD5^-$ B-cell chronic lymphocytic leukemia. Blood 86, 3883.

Malynn, B.A., Yancopoulos, G.D., Barth, J.E., Bona, C.A., Alt,

F.W., 1990. Biaised expression of JH-proximal VH genes occur in newly generated repertoire of neonatal and adult mice. J. Exp. Med. 171, 843.

Manoussakis, M.N., Tzioufas, A.G., Silis, M.P., Pange, P.J.E., Goudevenos, J., Moutsopoulos, H.M., 1987. High prevalence of anti-cardiolipin and other autoantibodies in a healthy elderly population. Clin. Exp. Immunol. 69, 557.

Marchalonis, J.J., Kaymaz, H., Dedeoglu, F., Schlutter, S.F., Yocum, D.E., Edmundson, A.B., 1992. Human autoantibodies reactive with synthetic autoantigens from T-cell receptor ß chain. Proc. Natl. Acad. Sci. U.S.A. 89, 3325.

Marchalonis, J.J., Kaymaz, H., Schluter, S.F., Yocum, D.E., 1994. Naturally occurring human autoantibodies to defined T-cell receptor and light chain peptides. Adv. Exp. Med. Biol. 347, 135.

Marcos, M.A., Sundblad, A., Malenchere, E., Coutinho, A., 1991. Peritoneal B cells regulate the numbers of allotype-matched pre-B and B cells in bone marrow. Proc. Natl. Acad. Sci. U.S.A. 88, 9944.

Mariotti, S., Sansoni, P., Barbesino, G., et al., 1992. Thyroid and other organ-specific autoantibodies in healthy centenarians. Lancet 339, 1506.

Martin, R., Jaraquemada, D., Flerlage, M., et al., 1990. Fine specificity and restriction of myelin basic protein specific cytotoxic T cell lines from multiple sclerosis patients and healthy controls. J. Immunol. 145, 540.

Martin, T., Crouzier, R., Weber, J., Kipps, T., Pasquali, J., 1994. Structure-function studies on a polyreactive (natural) autoantibody. Polyreactivity is dependent on somatically generated sequences in the third complementarity-determinig region of the antibody heavy chain. J. Immunol. 152, 5988.

Mendlovic, S., Brocke, S., Shoenfeld, Y., et al., 1988. Induction of a systemic lupus erythematosus-like disease in mice by a common human anti-DNA idiotype. Proc. Natl. Acad. Sci. U.S.A. 85, 2260.

Michael, J.G., 1969. Natural antibodies. Curr. Top Microbiol. Immunol. 48, 43.

Michel, C., Gonzalez, R., Bonjour, E., Avrameas, S., 1990. A concurrent increasing of natural antibodies and enhancement of resistance of furonculosis in rainbow trout. Ann. Rech. Vet. 21, 211.

Miletic, V.D., Hester, C.G., Frank, M.M., 1996. Regulation of complement activity by immunoglobulin. J. Immunol. 156, 749.

Miller, D.J., Rodriguez, M., 1995. A monoclonal autoantibody that promotes central nervous system remyelination in a model of multiple sclerosis is a natural autoantibody encoded by germline immunoglobulin genes. J. Immunol. 154, 2460.

Moiola, L., Karachunski, P., Protti, M.P., Howard, J.F., Conti-Tronconi, B.M., 1994. Epitopes of the b subunit of human muscle acetylcholine receptor by $CD4^+$ T cells of myasthenia gravis patients and healthy subjects. J. Clin. Invest. 93, 1020.

Moulias, R., Proust, J., Wang, A., et al., 1984. Age-related increase in autoantibodies. Lancet i, 1128.

Mouthon, L., Haury, M., Lacroix-Desmazes, S., Barreau, C., Coutinho, A., Kazatchkine, M.D., 1995a. Analysis of the normal human IgG antibody repertoire. Evidence that IgG autoantibodies of healthy adults recognize a limited and conserved set of protein antigens in homologous tissues. J. Immunol. 154, 5769.

Mouthon, L., Nobrega, A., Nicolas, N., et al., 1995b. Invariance and restriction towards a limited set of self-antigens characterize neonatal IgM antibody repertoires and prevail in autoreactive repertoires of healthy adults. Proc. Natl. Acad. Sci. U.S.A. 92, 3839.

Mouthon, L., Lacroix-Desmazes, S., Nobrega, A., Barreau, C., Coutinho, A., Kazatchkine, M.D., 1996. The self-reactive antibody repertoire of normal human serum IgM is acquired in early childhood and remains stable throughout life. Scand. J. Immunol. 44, 243.

Muryoi, T., Sasaki, T., Harata, N., Takai, O., Tamate, E., Yoshinaga, K., 1988. Heterogeneity of anti-idiotypic antibodies to anti-DNA antibodies in humans. Clin. Exp. Immunol. 71, 67.

Naito, N., Saito, K., Hosoya, T., et al., 1990. Anti-thyroglobulin autoantibodies in sera from patients with chronic thyroiditis and from healthy subjects: differences in cross-reactivity with thyroid peroxidase. Clin. Exp. Immunol. 80, 4.

Nakamura, M., Burastero, S.E., Notkins, A.L., Casali, P., 1986. Human monoclonal rheumatoid factor-like antibodies from CD5 $(Leu\text{-}1)^+$ B cells are polyreactive. J. Immunol. 140, 4180.

Nakamura, M., Burastero, S.E., Ueki, Y., Larrick, J.W., Notkins, A.L., Casali, P., 1988. Probing the normal and autoimmune B cell repertoire with Epstein-Barr virus: frequency of B cell producing monoreactive high affinity autoantibodies in patients with Hashimoto's disease and systemic lupus erythematosus. J. Immunol. 141, 4165.

Navin, T., Krug, E., Pearson, R., 1989. Effect of immunoglobulin M from normal human serum on Leishmania donovani promastigote agglutination, complement-mediated killing and phagocytosis by human monocytes. Infect. Immun. 57, 1343.

Nickerson, K.G., Berman, J., Glickman, E., Chess, L., Alt, F.W., 1989. Early human IgH gene assembly in Epstein-Barr virus transformed fetal B cells studies. Preferential utilization of the most JH-proximal D segment (DQ52) and two unusual VH-related rearrangements. J. Exp. Med. 169, 1391.

Nicoletti, A., Kaveri, S.V., Caligiuri, G., Bariety, J., Hansson, G., 1998. Immunoglobulin treatment reduces atherosclerosis in apoE knockout mice. J. Clin. Invest., in press.

Nobrega, A., Haury, M., Grandien, A., Malanchère, E., Sunblad, A., Coutinho, A., 1993. Global analysis of antibody repertoires. II. Evidence for specificity, self-selection and the immunological 'homunculus' of antibodies in normal serum. Eur. J. Immunol. 23, 2851.

Nobrega, A., Haury, M., Gueret, R., Coutinho, A., Weksler, M., 1996. The age-associated increase in autoreactive immunoglobulins reflects a quantitative increase in specifici-

ties detectable at lower concentrations in young mice. Scand. J. Immunol. 44, 437.

Nobrega, A., Malanchère, E., Hecker, L., Haury, M., Grandien, A., Coutinho, a.A., 1997. Diversity of antibody repertoire. I. Clonal frequencies of reactivities and patterns of complex clonal mixtures on immunoblots of natural antigens. Eur. J. Immunol.

Pashov, A., Kaveri, S., Kazatchkine, M.D., Bellon, B., 1996. Suppression of experimental autoimmune encephalomyelitis by intravenous immunoglobulin. In: Kazatchkine, M.D., Morell, A. (Eds.), Intravenous Immunoglobulin and Therapy. Parthenon, New York, p. 317.

Pashov, A., Dubey, C., Kaveri, S.V., Lectard, B., Huang, Y., Kazatchkine, M.D., Bellon, B., 1998. Normal immunoglobulin G protects against experimental allergic encephalomyelitis by inducing transferable T cell unresponsiveness to myelin basic protein. Eur. J. Immunol, in press.

Pechaczyk, M., Bouanani, M., Salhi, S.L., Baldet, L., Bastide, M., Pau, B., Bastide, J.M., 1987. Antigenic domains on the human thyroglobulin molecule recognized by autoantibodies in patient's sera and by natural autoantibodies isolated from sera of healthy subjects. Clin. Immunol. Immunopathol. 45, 114.

Pereira, P., Forni, L., Larsson, E.L., Cooper, M.D., Heusser, C., Coutinho, A., 1986. Autonomous activation of B and T cells in antigen-free mice. Eur. J. Immunol. 16, 685.

Pfueller, S.L., Logan, D., Tran, T.T., Bilston, R.A., 1990. Naturally occurring human IgG antibodies to intracellular and cytoskeletal components of human platelets. Clin. Exp. Immunol. 79, 367.

Poutsiaka, D.D., Clark, B.D., Vannier, E., Dinarello, C.A., 1991. Production of IL-receptor antagonist and IL-1ß by peripheral blood mononuclear cells is differentially regulated. Blood 78, 1275.

Prabhakar, B.S., Saegusa, J., Onodera, T., Notkins, A.L., 1984. Lymphocytes capable of making monoclonal antibodies that react with multiple organs are a common feature of the normal B cell repertoire. J. Immunol. 133, 2815.

Raaphorst, F.M., Timmers, E., Kenter, M.J.H., Van Tol, M.J.D., Vossen, J.M., Schuurman, R.K.B., 1992. Restricted utilization of germ-line Vh3 genes and short diverse third complementarity-determining regions (CDR 3) in human fetal B lymphocyte immunoglobulin heavy chain rearrangements. Eur. J. Immunol. 22, 247.

Reth, M.G., Jackson, S., Alt, F.W., 1986. VhDJH formation and DJH replacement during pre-B cell differentiation: non-random usage of gene segments. EMBO J. 5, 2131.

Ronda, N., Haury, M., Nobrega, A., Kaveri, S.V., Coutinho, A., Kazatchkine, M.D., 1994. Analysis of natural and disease-associated autoantibody repertoires: anti-endothelial cell IgG autoantibody activity in the serum of healthy individuals and patients with systemic lupus erythematosus. Int. Immunol. 6, 1651.

Rossi, F., Dietrich, G., Kazatchkine, M.D., 1989. Anti-idiotypes against autoantibodies in normal immunoglobulins: evidence for network regulation of human autoimmune responses. Immunol. Rev. 110, 135.

Rossi, F., Kazatchkine, M.D., 1989. Antiidiotypes against autoantibodies in pooled normal human polyspecific Ig. J. Immunol. 143, 4104.

Rossi, F., Guilbert, B., Tonnelle, C., et al., 1990. Idiotypic interactions between normal human polyspecific IgG and natural IgM antibodies. Eur. J. Immunol. 20, 2089.

Rossi, F., Bellon, B., Vial, M.C., Druet, P., Kazatchkine, M.D., 1991a. Beneficial effect of human therapeutic intravenous immunoglobulins (IVIg) in mercuric-chloride-induced autoimmune disease of Brown-Norway rats. Clin. Exp. Immunol. 84, 129.

Rossi, F., Jayne, D.R.W., Lockwood, C.M., Kazatchkine, M.D., 1991b. Anti-idiotypes against anti-neutrophil cytoplasmic antigen autoantibodies in normal human polyspecific IgG for therapeutic use and in the remission sera of patients with systemic vasculitis. Clin. Exp. Immunol. 83, 298.

Roux, K.H., Tankersley, D.L., 1990. A view of the human idiotypic repertoire: electron microscopic and immunologic analyses of spontaneous idiotype-anti-idiotype dimers in pooled human IgG. J. Immunol. 144, 1387.

Rudensky, A.Y., Mazel, S.M., Yurin, V.L., 1990. Presentation of endogenous immunoglobulin determinants to immunoglobulin-recognizing T cell clones by the thymic cells. Eur. J. Immunol. 20, 2235.

Ruiz-Arguelles, A., 1988. Spontaneous reversal of acquired autoimmune dysfibrinogenemia probably due to an anti-idiotypic antibody directed to an interspecies cross-reactive idiotype expressed on anti-fibrinogen antibodies. J. Clin. Invest. 82, 958.

Ruiz de Souza, V., Carreno, M.P., Kaveri, S.V., et al., 1995. Selective induction of interleukin-1 receptor antagonist and interleukin-8 in human monocytes by normal polyspecific IgG (intravenous immunoglobulin). Eur. J. Immunol. 25, 1267.

Sabbaga, J., Pankewycz, O.G., Lufft, V., Schwartz, R.S., Madaio, M.P., 1990. Cross-reactivity distinguishes serum and nephritigenic anti-DNA antibodies in human lupus from their natural counterparts in normal serum. J. Autoimmun. 3, 215.

Saenko, V.A., Kabakov, A.E., Poverenny, A.M., 1992. Hidden high-avidity anti-DNA antibodies occur in normal human gammaglobulin preparations. Immunol. Lett. 34, 1.

Sanz, I., Dang, H., Takei, M., Talal, N., Capra, J.D., 1989. Vh sequence of a human anti-Sm autoantibody: Evidence that autoantibodies can be unmutated copies of germline genes. J. Immunol. 142, 883.

Saoudi, A., Hurez, V., de Kozak, Y., et al., 1993. Human immunoglobulin preparations for intravenous use prevent experimental autoimmune uveoretinitis. Int. Immunol. 5, 1559.

Schettino, E.W., Ichiyoshi, Y., Casali, P., 1996. Structure-function relation in natural and disease-associated human autoantibodies. In: Zanetti, M., Capra, J.D. (Eds.), The Antibodies. Harwood Academic Publishers, Amsterdam, p. 155.

Schroeder, H.W., Wang, J.Y., 1990. Preferential utilization of conserved immunoglobulin heavy chain variable gene seg-

ments during human fetal life. Proc. Natl. Acad. Sci. U.S.A. 87, 6146.
Schroeder, H.W., Hillson, J.L., Perlmutter, P.M., 1987. Early restriction of the human antibody repertoire. Science 238, 791.
Schutte, M.E.M., Ebeling, S.B., Akkermans, K.E., Gmelig-Meyling, F.H.J., Logtenberg, T., 1991. Antibody specificity and immunoglobulin Vh gene utilization of human monoclonal CD5$^+$ B cell lines. Eur. J. Immunol. 21, 1115.
Spalter, S.H., Kaveri, S., Mani, J.C., Kazatchkine, M.D., 1997. Natural antibodies of the IgG and IgM isotypes reactive with self ABO blood group antigens are present in normal human serum, submitted.
Stewart, J., 1992. Immunoglobulins did not arise in evolution to fight infection. Immunol. Today 13, 396.
Sultan, Y., Kazatchkine, M.D., Maisonneuve, P., Nydegger, U.E., 1984. Anti-idiotypic suppression of autoantibodies to Factor VIII (antihaemophilic factor) by high-dose intravenous gammaglobulin. Lancet ii, 765.
Sultan, Y., Rossi, F., Kazatchkine, M.D., 1987. Recovery from anti-VIII:C (antihemophilic factor) autoimmune disease is dependent on generation of antiidiotypes against anti-VIII:C autoantibodies. Proc. Natl. Acad. Sci. U.S.A. 84, 828.
Sundblad, A., Marcos, M., Huetz, F., et al., 1991. Normal serum immunoglobulins influence the number of bone marrow pre-B and B cells. Eur. J. Immunol. 21, 1155.
Sundblad, A., Marcos, M., Malenchere, E., et al., 1994. Observations on the mode of action of normal immunoglobulin at high-doses. Immunol. Rev. 139, 125.
Svenson, M., Hansen, M.B., Bendtzen, K., 1990. Distribution and characterization of autoantibodies to interleukin 1 α in normal human sera. Scand. J. Immunol. 32, 695.
Talor, E., Rose, N.R., 1991. Hypothesis: the aging paradox and autoimmune disease. Autoimmunity 8, 245.
Tankersley, D.L., Preston, M.S., Finlayson, J.S., 1988. Immunoglobulin G dimer: an idiotype-anti-idiotype complex. Mol. Immunol. 25, 41.
Ternynck, T., Avrameas, S., 1986. Murine natural monoclonal autoantibodies: a study of their polyspecificities and their affinities. Immunol. Rev. 94, 99.
Thornton, B.P., Vetvicka, V., Ross, G.D., 1994. Natural antibody and complement-mediated antigen processing and presentation by B lymphocytes. J. Immunol. 152, 1727.
Tighe, H., Chen, P.P., Tucker, R., et al., 1993. Function of B cells expressing a human immunoglobulin M rheumatoid factor autoantibody in transgenic mice. J. Exp. Med. 177, 109.
Tomer, Y., Shoenfeld, Y., 1988. Aging and autoantibodies. Autoimmunity 1, 141.
van Doorn, P.A., Rossi, F., Brand, A., van Lint, M., Vermeulen, M., Kazatchkine, M.D., 1990. On the mechanism of high dose intravenous immunoglobulin treatment of patients with chronic inflammatory demyelinating polyneuropathy. J. Neuroimmunol. 29, 57.
Varela, F., Coutinho, A., 1991. Second generation immune networks. Immunol. Today 12, 159.
Vassilev, T., Gelin, C., Kaveri, S.V., Zilber, M.T., Boumsell, L., Kazatchkine, M.D., 1993. Antibodies to the CD5 molecule in normal human immunoglobulins for therapeutic use (intravenous immunoglobulins IVIg). Clin. Exp. Immunol. 92, 369.
Vassilev, T., Bineva, I., Dietrich, G., Kaveri, S.V., Kazatchkine, M.D., 1995. Variable region-connected, dimeric fraction of intravenous immunoglobulin enriched in natural autoantibodies. J. Autoimmun. 8, 405.
Vassilev, T., Kazatchkine, M.D., Mani, J.C., Lecroubier, C., Schriever, F., Kaveri, S.V., 1997. Antibodies to to the RGD (Arg-Gly-Asp) Peptide, Capable of Inhibiting Cellular Adhesion Interactions, Are Present in Normal Immunoglobulin for Therapeutic Use (Intravenous immunoglobulin, IVIg), in preparation.
Weir, D.M., Elson, C.J., 1969. Antitissue antibodies and immunological tolerance to self. Arthritis Rheum. 12, 254.
Wilson, G.S., Miles, A.A., 1964. The natural antibodies: their nature, origin and behaviour. In: Wilson, T.A. (Ed.), Principle of Bacteriology and Immunity. Edward Arnold, London, p. 1315.
Witz, I., Yaakubowicz, M., Gelernter, I., et al., 1984. Studies on the level of natural antibodies reactive with various tumor cells during urethane carcinogenesis in BALB/c mice. Immunobiology 166, 131.
Xu, C., Poirier, B., Lavaud, S. et al., 1997. Normal polyspecific human immunoglobulins IVIg modulate endothelial cell function in vitro, in preparation.
Yadin, O., Sarov, B., Naggan, L., Slor, H., Shoenfeld, Y., 1989. Natural autoantibodies in the serum of healthy women — a five year follow-up. Clin. Exp. Immunol. 75, 402.
Ye, Y.L., Chuang, Y.H., Chiang, B.L., 1996. In vitro and in vivo functional analysis of CD5$^+$ and CD5$^-$ B cells of autoimmune NZB x NZW F1 mice. Clin. Exp. Immunol. 106, 253.
Youinou, P.Y., Mackenzie, L., Masson, G.L., et al., 1988. CD5-Expressing B lymphocytes in the blood and salivery glands of patients with Primary Sjögren's syndrome. J. Autoimmun. 1, 185.
Zouali, M., Eyquem, A., 1983. Idiotypic/antiidiotypic interactions in systemic lupus erythematosus: demonstration of oscillary levels of anti-DNA autoantibodies and reciprocal antiidiotypic activity in a single patient. Ann. Inst. Pasteur Immunol. 134C, 377.