AUTOANTIBODIES OF VARIOUS SPECIFICITIES ENCODED BY GENES FROM THE V_{H} J558 FAMILY BIND TO FOREIGN ANTIGENS AND SHARE IDIOTOPES OF ANTIBODIES SPECIFIC FOR SELF AND FOREIGN ANTIGENS

BY MARC MONESTIER,* BEATRICE BONIN*, PAOLA MIGLIORINI, HOWARD DANG, SYAMAL DATTA, RUDOLF KUPPERS, NOEL ROSE, PAUL MAURER, NORMAL TALAL, AND CONSTANTIN BONA*

From the *Department of Microbiology, Mount Sinai School of Medicine, New York 10029; the 1Department of Medicine, Tufts University/New England Medical Center, Boston, Massachusetts 02111; the 2Department of Medicine, Division of Clinical Immunology, University of Texas, San Antonio, Texas 78212; 1The School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland 21218; and 1The Department of Biochemistry, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

After the formulation of the clonal selection theory by Burnet (1) and the discovery of idiotypes by Kunkel (2) and Oudin (3), immunology was dominated by the concept that one clone produces one antibody expressing one antigenic marker (idiotype) and recognizing one antigenic determinant (epitope). This paradigm was supported by data suggesting a triple relationship between the sequence of the hypervariable region, specificity of the combining site, and idiotope (4).

This concept was challenged by two unexpected observations: that the same idiotope can be shared by antibodies with various specificities (5), and that a myeloma protein can bind to two different antigens (i.e., MOPC460, which binds to dinitrophenyl hapten and menadione) (6).

Later, we described another category of multispecific antibodies, called epitopes, which bind to idiotopes and to autoantigens (7). Such antibodies can connect the repertoire for foreign antigens and self antigens; Dwyer et al. (8) have shown that certain antidextran antibodies also bind to anti-acetylcholine receptor antibodies via idiotypic interactions.

Recent studies provided numerous examples of multispecific autoantibodies. Thus, it was shown that human monoclonal proteins with rheumatoid factor properties can bind to histones (9), that thymic B lymphocytes from patients with myasthenia gravis secrete mAbs that bind to myosin, α-actinin, and actin (10), and that a high proportion of mAbs obtained from early B cells exhibit multiple self reactivities (11). It was also shown that a human monoclonal macroglobulin with specificity for α(2,8)-linked poly-N-acetyl neuraminic acid binds to polynucleotides or denatured DNA (12), or that mAbs obtained from mice immunized

This work was supported by U.S. Public Health Service grant A118316-94 from the National Institute of Aging. M. Monestier was supported by a fellowship from Merck, Sharp, and Dohme, Inc., and by a grant from the Lupus Foundation of America, Inc.
with phenylarsonate (13) or *Streptococcus pyogenes* M type 5 (14) bind to DNA, cytoskeleton, myosin, or keratin, respectively.

In this study we addressed two questions: Do "bona fide" autoantibodies obtained from autoimmune mice or from animals immunized with autoantigens in complete Freund's adjuvant (CFA) bind to foreign antigens? Do autoantibodies with a given specificity share idiotopes with autoantibodies of different specificities and with antibodies specific for foreign antigens?

We found that it is important to address these questions because it is not clearly known if the expression of self-reactive clones is driven by autoantigens or exogenous antigens.

Our study was carried out on antibodies encoded by genes from the V$_a$ J558 family selected from our panel of 63 mAbs (15).

The choice of this group of autoantibodies was made for several reasons: (a) We possess a large panel of foreign antigens known to bind to antibodies encoded by genes from the V$_a$ J558 family. (b) Victor-Kobrin et al. (16) have previously shown that there is a high connectivity among antibodies with various specificities encoded by V$_a$ genes from this family. (c) We possess multiple crossreactive idiotypic systems expressed on V$_a$ J558+ antibodies specific for foreign and self-reactive antigens.

The results presented in this communication show that 9 of 20 autoantibodies also bind to foreign antigens and that they share idiotopes with antibodies specific for autoantigens or foreign antigens.

**Materials and Methods**

**Antigens.** The panel of antigens used in this study is illustrated in Table I. They are known to bind to murine antibodies encoded by V genes derived from the V$_a$ J558 family (17).

Cardiolipin, thyroglobulin and hen egg white lysozyme were purchased from Sigma Chemical Co., St. Louis, MO. Phenylarsonate and nitrophenyl acetate conjugates were a gift from Drs. M. Gefter and T. Imanishi (Massachusetts Institute of Technology, Cambridge, Massachusetts), and dextran antigens were from Dr. E. Kabat (Columbia University, New York). Synthetic antigens were obtained according to a previously described technique (18), and the lipopolysaccharides of our own collection were extracted from various gram-negative bacteria by the method of Westphal et al. (19).

**Monoclonal Antibodies**

The origin, specificity, and isotypes of the mAbs used in this study are shown in Table II. All of these autoantibodies have been previously identified as using a V$_a$ gene derived from V$_a$ J558 family (15).

**Binding to Antigens**

The binding of the V$_a$ J558+ autoantibodies to various antigens was determined by radioimmunoassay (RIA). Microtiter plates were coated overnight at 4°C with 10 μg/ml antigen. After washing and postcoating with BSA, the plates were incubated for 2 h at 20°C with various concentrations of antibodies. After extensive washings, plates were incubated for 2 h at 20°C with $^{125}$I-labeled rat anti-mouse κ mAb (50,000 cpm/well), washed extensively, and the radioactivity was counted in a gamma counter.

Specificity of the binding was studied by using a competitive inhibition RIA. This technique was carried out in two steps: (a) in liquid phase, 0.5 μg of antibody were incubated with various amounts of antigens (15 and 150 ng) for 2 h at room temperature in microtubes coated previously with BSA, and (b) 50 μl of this mixture was transferred to microplates coated with antigens as described above.
TABLE I

Foreign Antigens Used in This Study

| Source or type          | Antigens                                                                 |
|-------------------------|--------------------------------------------------------------------------|
| α1-3, 1-6 Dextran        | B1948L, B1355S, B512, B1254S, B1510S, B1375, B1424, B1141, B1425, B1355L, B1498S, B742S, B1399, B1501L, B742L, B1142, B1299SB, B1255 |
| Influenza virus          | PR8(H1N1), X31(H3N2), B/Lee, A Singapore (H1N1)                          |
| Synthetic peptides       | Poly(Gluα, Lys10, Ala60)(GLA60)                                          |
|                         | Poly(Gluα, Lys57, Phe60)(GLP)                                           |
|                         | Poly(Gluα37, Tyr60)(GT)                                                 |
|                         | Poly(Gluα, Phe60)(Gφ)                                                  |
|                         | Poly(Gluα, Lys60)(GL)                                                  |
|                         | Poly(Gluα, Ala60, Tyr60)(GAT)                                           |
| Lyopolysaccharides       | Proteus morganii 4B III                                                 |
|                         | Escherichia coli 0113                                                  |
|                         | Proteus vulgaris 5CIII                                                  |
|                         | Providencia stuartii 12A X                                              |
|                         | Salmonella minnesota R 595                                              |
|                         | Klebsiella oxytoca 9B IV                                               |
|                         | S. transoara                                                           |
|                         | Proteus mirabilis 8A III                                                |
|                         | Bacteroides fragilis                                                   |
|                         | S. thompson S-PP-385                                                   |
|                         | Pseudomonas aeruginosa                                                 |
|                         | Klebsiella pneumoniae 7A IV                                             |
|                         | S. anatum S-PP-385                                                     |
|                         | E. coli 0111 27C XI                                                    |
|                         | S. newington                                                           |
|                         | Neisseria lactamica 26A XIV                                             |
|                         | Enterobacter cloaca 10A VII                                             |
|                         | Shigella dysenteriae 20C VIII                                           |
|                         | Serratia marcescens 6 AV                                               |
|                         | Pseudomonas fluorescens 2C II                                           |
|                         | E. coli K235                                                           |
| Haptens                 | Nitrophenyl acetate–chicken IgG, phenylarsonate-BSA                     |
| Protein antigens        | Hen lysozyme                                                           |

Affinity Measurement

Affinity measurements were carried out as described by Friguet et al. (20). Briefly, in a preliminary experiment, each antibody at various concentrations was incubated for different lengths of time in microtiter plates coated with the antigen. The content of each well was transferred into another coated well and incubated for the same time. In the two series of wells, the bound antibody was revealed using 125I-labelled anti-α as described before. This procedure was performed to determine that no readjustment of the equilibrium in the liquid phase would occur during the experiment aimed to measure affinity. We considered a time period satisfactory when the binding in the second set of wells was not less than 85% of the binding observed with the first set. The affinity was measured using an RIA similar to the one described previously. Antibody at a known concentration was incubated overnight with various amounts of antigen in PBS-BSA. The antibody-
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**Table II**
Characteristics of Autoantibodies Used in This Study

| Designation | Origin | Specificity | Isotype | \(V_{\mu}\) | Reference |
|-------------|--------|-------------|---------|-------------|-----------|
| 10VA2       | CBA/J mice immunized with        |             | \(\mu k\) | J558        | 42        |
| 84 A3       | TG     | Thyroglobulin| \(\mu k\) | J558        | 42        |
| 81 B1       |        |             | \(\mu k\) | J558        | 42        |
| 81 D2       |        |             | \(\gamma 2bk\) | J558 | 42        |
| H102        | MRL/lpr spontaneous              |             | \(\gamma 2ak\) | J558 | 42        |
| H130        |        | DNA         | \(\mu k\) | J558        | 42        |
| H241        |        |             | \(\gamma 2ak\) | J558 | 42        |
| E8          | DBA/1 immunized with type II collagen | Type II collagen | \(1\)k | J558 | 44       |
| A12         |        |             | \(\gamma 2bk\) | J558 | 44       |
| Y2          | MRL/lpr spontaneous              | Sm          | \(\gamma 2ak\) | J558 | 23       |
| Y12         |        |             | \(\gamma 2ak\) | J558 | 23       |
| 6B6         |        |             | \(\mu k\) | J558        | 23       |
| 15–32       | SJL immunized with MBP           | Myelin basic protein | \(\gamma 2ak\) | J558 | 45       |
| S2-9-2      | Motheaten spontaneous            |             | \(\mu k\) | J558        | 45       |
| UN59-9      |        |             | \(\mu k\) | J558        | 45       |
| 6-19-23     | MRL/lpr spontaneous              |             | \(\gamma 3k\) | J558 | 46       |
| MRL50-8     |        |             | \(\mu k\) | J558        | 46       |
| Y19-10      | BALB/c immunized with Y. enterocolitica | Rheumatoid factor | \(\mu k\) | J558 | 22       |
| Y19-16      |        |             | \(\mu k\) | J558        | 22       |
| LPS5-4      | BALB/c in vitro LPS stimulation  |             | \(\mu k\) | J558        | 22       |

The antigen mixture was then transferred into antigen-coated wells and incubated for the time determined in the preliminary experiment. The binding was revealed by \(^{125}\text{I}-\text{anti-}\kappa\) antibody. The calculations for the affinity determination were performed according to Friguet et al. (20) and the \(K_d\) is expressed in grams per milliliter instead of molar because of the nature and the source of the antigens used (varying molecular weights and heterogeneity of polymers). The same expression of \(K_d\) was used by Sharon et al. (21) to measure the affinity of antibodies specific for dextran, a complex natural antigen.

**Study of Idiotyp**

Two groups of idiotypic systems were used in this study, defining crossreactive idiotypes (IdX)\(^1\) of autoantibodies and of antibodies specific for foreign antigens. These idiotypes are expressed on antibodies encoded by \(V_{\mu}\) J558 family-derived \(V_{\mu}\) genes.

**Idiotypes of Autoantibodies.** Y19-10 anti-LPS 10-1 defines a crossreactive idiotype recognized by polyclonal rabbit antibodies produced by immunization with LPS 10-1, which is a BALB/c mAb exhibiting rheumatoid factor (RF) activity (22). These rabbit anti-Id antibodies recognize an IdX on Y19-10, a monoclonal RF obtained from a BALB/c mouse immunized with *Yersinia enterocolitica* (22).

Anti-Y2Id is a rabbit anti-Id antibody raised against Y2, an anti-Sm mAb obtained from MRL/lpr mouse (23).

\(^1\)Abbreviations used in this paper: Ars, p-azophenyl arsonate; IdX, crossreactive idiotype; RF, rheumatoid factor.
H130 is a monoclonal anti-DNA antibody obtained from MRL/1pr mouse and 108, an anti-Id mAb against H130 (24).

Y19-10, LPS10-1, Y2, and H130 use genes derived from the V\textsubscript{H} J558 family (15).

**Idiotypes of Antibodies Specific for Foreign Antigens.** J558-CD3-2 defines J558 IdX expressed on a majority of α-1,3-dextran antibodies. CD3-2 is a previously described monoclonal anti-J558 IdX antibody, kindly donated by Dr. J. Kearney (Univ. of Alabama, Birmingham, AL) (25).

G5 is an mAb specific for the GAT terpolymer. HP20 is an mAb recognizing a GAT-crossreactive idiotype (26). Both reagents are a gift from Dr. M. Fougereau (Centre Inserm, Marseille-Luminy, France).

The IdX of antiarsenite (anti-Ars) antibodies was defined by 36-65-AD8, mAbs previously described (27). 36-65, an anti-Ars mAb is a gift from Dr. M. Gefter (MIT, Cambridge, MA), and AD8 is from Dr. G. Lewis (University of Maryland, Baltimore, MD).

PY211 is an mAb specific for PR8 influenza virus hemagglutinin and 63.4, a syngeneic mAb recognizing a crossreactive idiotype on Py211 (28).

PY206 is an mAb specific for X31 influenza virus hemagglutinin and SN3.9A, a syngeneic mAb recognizing a crossreactive idiotype on PR8- and X31-specific antibodies (28).

IDA23 is an anti-A48-Id mAb and AIDA23, a syngeneic anti(anti-Id) mAb. These reagents were kindly donated by P. Legrain (Pasteur Institute, Paris, France).

J558, G5, 36-65, PY211, PY206, and IDA23 are mAbs encoded by V\textsubscript{H} genes from the V\textsubscript{H} J558 family (26, 27, 28, 29).

Presence of crossreactive idiotypes was determined by a competitive inhibition RIA. Briefly, microtiter plates were coated overnight at 4°C with chromatographically purified anti-Id antibodies (10 µg/ml) in carbonate buffer, pH 9.2. After washing and postcoating with PBS-BSA, the plates were incubated with the antibodies to be tested (10 µg/ml in PBS-BSA). After 2 h at 20°C, the plates were washed and incubated with 50,000 cpm of the corresponding idiotype labelled with \textsuperscript{125}I. After extensive washing, radioactivity was counted using a gamma counter.

The specificity of the idiotypic systems shared by J558+ antibodies directed against foreign antigens was determined by using mAbs, prototypes of each of the seven major murine V\textsubscript{H} families.

**Results**

**Binding to Foreign Antigens.** The binding activities of 20 V\textsubscript{H} J558+ autoantibodies with various specificities were tested against both the corresponding autoantigen and all foreign antigens, included our panel (Table I). 9 of 20 autoantibodies studied exhibited various degrees of binding to foreign antigens. The data presented in Fig. 1 show a dose effect relationship of binding for 15-32, H102, H130, and UN59-9, whereas a binding by high doses of antibody (3 and 10 µg/ml) was observed with the other antibodies. The competitive inhibition assay was carried out to determine whether or not the binding to auto- and foreign antigens is an intrinsic property of the antibody-combining site. The data presented in Table III show that the binding of autoantibody to the corresponding autoantigen was antigen-inhibitable (excepting 15-32, for which the binding to GLϕ was not inhibited by MBP). Similarly, the binding to foreign antigen by autoantibody was inhibited by the corresponding autoantigen as well as by the foreign antigen. We also examined the binding to phosphocholine, levan, inulin, and TNP. These antigens are known to bind to antibodies encoded by V genes from the V\textsubscript{H} S107, V\textsubscript{H} X24, V\textsubscript{H} J606, and V\textsubscript{H} 36-60 families, respectively (17). No binding to these antigens by our V\textsubscript{H} J558+ autoantibodies that bound to foreign antigens was observed (data not shown).
We determined the affinity of binding of the 9 out of 20 antibodies that showed crossreactivity with foreign antigens. The data presented in Table IV show that the $K_d$ is usually lower for autoantigens than for foreign antigens, with the exception of Sm-specific autoantibodies (6B6 and Y12). It should be mentioned that because of the particularly complex nature of the antigen used, the $K_d$ were expressed as grams per milliliter, as was done by Sharon et al. (21) with antidextran antibodies.

We also investigated the binding to GT, GAT, and GLψ of a panel of 30 autoantibodies encoded by V genes from $\nu Q52$ and $\nu 7183$ families (Table V). We did not observe significant binding of these antibodies, with the exception of a monoclonal anti-DNA antibody (11112), which showed binding to GT.

**Study of Expression of Crossreactive Idiotypes.** In further experiments, we examined our $\nu J558^*$ autoantibodies for the presence of IdX originally borne by autoantibodies and by antibodies specific for foreign antigens and encoded by $\nu J558$ genes. There are three reasons for our study: First, we have shown the existence of a high idiotypic connectivity among autoantibodies of various specificities (18). Second, we found that 9 of our 20 autoantibodies bind to foreign epitopes and, since IdX are markers of germline genes (30), the presence of IdX common to autoantibodies and antibodies specific for foreign antigens may indicate a common germline origin. Third, the presence of IdX among autoantibodies and antibodies specific for foreign antigen can shed light on the activation mechanism of self-reactive clones, since it is known that anti-Id antibodies can influence the expansion of autoreactive clones (reviewed in 31).

The summary results of these studies are shown on Table VI and were determined by RIA in a dose-response manner: 15 of 20 antibodies express the
TABLE III

Inhibition of Binding of Autoantibodies to Self Antigens by Foreign Antigens

| mAb (10 ng/ml) | Binding to plates coated with: | Percent inhibition of binding with: * |
|---------------|-------------------------------|-------------------------------------|
|               | MBP | GLΦ | GT | Lysozyme | Sm | TG | G2a | DNA | Ars | E. coli | S. providencia |
| 15-32         | 6.3 | 61.7 | 0 | 72 |
| UNS9-9        | 55.5 | 83.6 | 86.5 | 54.6 |
|               | 77 | 88.1 | |
|               | 62.5 | 70.8 | |
| Lysosome      | 55 | 60.6 | |
| 6B6           | 58.2 | 50.9 | 55.3 | |
|               | 82.6 | 73.5 | |
|               | 86.1 | 80.7 | |
| 8B6           | 40.6 | 42.4 | 79 | |
| 8IB1          | 84.8 | 76.3 | 76.3 | 74.4 |
|               | 88.2 | 74.4 | |
| 81A3          | 80.5 | 80.6 | 62.3 | 66.7 |
|               | 81.9 | 52.5 | |
|               | 91.4 | 66.7 | |
| LP55-4        | 53.1 | 46.5 | 54.3 | |
|               | 66 | 70.1 | 59.6 | |
|               | 68.6 | 71.2 | 65.5 | |
| H130          | 78.4 | 85.8 | 60 | 50.9 | 67.6 |
| DNA           | 79.7 | 75.6 | 90.6 | 70 | 94.2 |
| Cardiolipin   | 52.9 | 20 | 86.3 | |
| Lysosome      | 85 | 58.3 | 84 | 85.5 |
| Ars           | |
| H102          | 77.2 | 75.8 | 65 | 66.5 | |
| DNA           | 44 | 66 | 91.7 | 74.5 |
| Cardiolipin   | 81.5 | 61 | |
| Ars           | 58.3 | 59.7 | |
| S. providencia| 58.7 | 70.8 | |

* Antigens used at 15 ng/well.

Idiotypic analysis

Idiotyped autoantibodies were used to determine the specificity of the idiotypic system. Seven purified antibody proteins, prototypes of major murine V ones, did not cause inhibition, except J558 in the J558-CD3-2 idiotypic system. The data presented in Table VIII summarize the results of this system, which show that 3 of 30 autoantibodies share the J558-IdX; 3 of 20 share the PY211 IdX, an anti-
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**Table IV**

| Antibody | Antigen | $K_d$ (g/ml) |
|----------|---------|-------------|
| 15-32    | MBP     | $5.2 \times 10^{-5}$ |
| UN59-9   | MBP     | $1.2 \times 10^{-4}$ |
|          | GT      | $9.4 \times 10^{-5}$ |
|          | GL\_\phi | $1.0 \times 10^{-5}$ |
|          | Lysozyme | $8.9 \times 10^{-5}$ |
| 6B6      | Sm      | $6.6 \times 10^{-5}$ |
|          | GT      | $7.6 \times 10^{-5}$ |
|          | GL\_\phi | $8.0 \times 10^{-5}$ |
| Y12      | Sm      | $1.8 \times 10^{-4}$ |
|          | GAT     | $6.5 \times 10^{-5}$ |
| 81D1     | TG      | $7.8 \times 10^{-5}$ |
|          | GT      | $7.8 \times 10^{-5}$ |
| 84A3     | TG      | $1.2 \times 10^{-4}$ |
|          | GT      | $7.0 \times 10^{-5}$ |
|          | GL\_\phi | $7.8 \times 10^{-5}$ |
| H130     | DNA     | $1.2 \times 10^{-4}$ |
|          | GL\_\phi | $6.6 \times 10^{-5}$ |
|          | GT      | $1.2 \times 10^{-4}$ |
|          | Lysozyme | $1.6 \times 10^{-5}$ |
|          | Ars     | $5.3 \times 10^{-5}$ |
| H102     | DNA     | $1.4 \times 10^{-5}$ |
|          | GT      | $5.3 \times 10^{-5}$ |
|          | E. coli | $5.2 \times 10^{-5}$ |
|          | S. providencia | $1.6 \times 10^{-4}$ |
| LPS5-4   | \(\gamma2\A) | $1.2 \times 10^{-4}$ |
|          | GT      | $4.5 \times 10^{-5}$ |
|          | GL\_\phi | $8.0 \times 10^{-5}$ |

influenza virus hemagglutinin antibody; 3 of 20 share an IdX present on G5, a GAT-specific antibody; and 3 of 20 share an IdX on 36-65, an anti-Ars antibody.

It should be mentioned that none of the autoantibodies encoded by V\_H QP52 and V\_H J558 family genes (Table V) expressed the IdX originally borne by V\_H J558\(^+\) antibodies directed against foreign antigens (data not shown).

**Discussion**

In this communication, we present results showing that autoantibodies obtained from animals prone to develop autoimmune disease, or animals actively immunized with autoantigen bind to foreign antigens and share idiotypes with autoantibodies of various specificities, as well as with antibodies specific for foreign antigens. In our study, we only used autoantibodies encoded by genes...
derived from the $V_h$ J558 family, since we possess a large panel of antigens known to bind to antibodies encoded by the same $V_h$ gene family. The binding studies to foreign antigens show that 9 of 20 antibodies bind with varying degrees to foreign antigens, and particularly to synthetic antigens such as GT and GLO. Two anti-DNA antibodies showed significant binding to other antigens: H130 to lysozyme and Ars, and H102 to *E. coli* and *S. providenciae*.

Competitive inhibition experiments demonstrated the specificity of this binding, and affinity measurements showed values commonly found in antigen/antibody interactions, even though the particular nature of some antigens and their lack of purity did not allow us to draw definite conclusions from this affinity measurement.

However, the $K_d$ of "bona fide" antibodies directed against foreign antigens was usually lower than the $K_d$ of multispecific antibodies. While the $K_d$ of various autoantibodies binding to GT varies between $1.2 \times 10^{-4}$ and $9.4 \times 10^{-5}$, G5, a

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### TABLE V

Autoantibodies with Various Specificities Sharing Crossreactive Idiotypes with Autoantibodies Encoded by $V_h$ J558 Family Genes

| $V_h$ families | Designation | Specificity |
|---------------|-------------|-------------|
| QPC52         | MRL5-51, LPS 7-3 | RF |
|               | 84D1B1      | TG |
|               | HB8         | Skin antigens |
| 7183          | B36, Z26, M93, M16 | Sm |
|               | Z317, Z121, Z41, Z49, HB2 | DNA |
|               | M88, B57, B61, B56, MRL22-46 | RF |
|               | LPS10-4, 129-48, 129-78 | |
|               | CP5, CP4    | RBC |
|               | Id62, 1-15, B10 H2 AD2 | TG |
|               | LE4         | TSH receptor |
|               | G2          | Collagen type II |
|               | HB10, HB12  | Skin antigens |

### TABLE VI

Autoantibodies with Various Specificities Sharing Crossreactive Idiotypes with Autoantibodies Encoded by $V_h$ J558 Family Genes

| Idiotypic system | Specificity of idiotype | Autoantibodies sharing crossreactive idiotypes | Frequency |
|------------------|-------------------------|-----------------------------------------------|-----------|
| Y19-10-anti-LPS10-1 | RF | Y19-10(RF), LPS5-4(RF), Y19-16(RF), MRL50-8(RF), Y12(Sm), 6B6(Sm), H102(DNA), H130(DNA), 15-32(MBP), 84A3(TG), 10VA2(TG), 81 B1(TG), 81D2(TG), UN59-9(MBP), S2-92(MBP) | 15/20 |
| Y2-anti-Y2        | Sm | Y-2(Sm), Y-12(Sm), 6B6(Sm), LPS5-4(RF), Y19-16(RF), 6-19-23(RF) | 6/20 |
| H130-anti-H130    | DNA | H130(DNA), H102(DNA), H241(DNA), 10VA2(TG), 81 B1(TG), 6B6(Sm) | 6/20 |
monoclonal anti-GT antibody, had a $K_d$ of $1.4 \times 10^{-7}$. The $K_d$ of H130 to arsonate ($5.3 \times 10^{-4}$) or to lysozyme ($1.6 \times 10^{-4}$) was also higher than the $K_d$ of 36-65 ($4.3 \times 10^{-6}$), an anti-Ars mAb or than the $K_d$ of four antilysozyme mAbs (1C8, $1.1 \times 10^{-7}$; 17, $5.7 \times 10^{-8}$; 2E5, $3.1 \times 10^{-8}$; and 2F4, $7.2 \times 10^{-8}$). The higher affinity of "bona fide" antibodies was expected because these clones have been expanded in vivo and selected in vitro by the corresponding antigen. However, small differences in the affinity of autoreactive clones for foreign antigens compared with the clones expanded by the same antigens suggest the possibility of the activation of autoreactive clones by foreign antigens.

Our data are in agreement with other findings that demonstrated that mAbs obtained from 6-d-old BALB/c mice exhibit a high frequency of self-reactivity, and that eight of these antibodies bound to TNP and self antigens such as actin, tubulin, and myosin (11). Guilbert et al. (32) have also shown that mice hyper-immunized with various antigens in CFA produced multispecific antibodies directed against foreign and autoantigens. Naparstek et al. (33) have shown that the unmutated $V_n$ IdCR gene, which encodes most of anti-Ars antibodies in A/J mice, also encodes antibodies binding to DNA and cytoskeletal proteins.

This crossreactivity for self and foreign antigens can be important in the breaking of self-tolerance. Patients with acute rheumatic fever have antibodies reacting with heart tissue (34), brain (35), and skeletal muscles (36). Because of the polyclonal nature of these antibodies, immunochemical studies aimed at defining their precise specificity have been difficult. However, these observations have been confirmed by studies using mAbs. Thus, mAb obtained from a BALB/c mouse and directed against S. pyogenes M type 5 also reacted with muscle proteins (14).

Taken collectively, these data suggest that autoreactive clones can be activated either directly by foreign antigens sharing epitopes with autoantigens, or by the intrinsic ability of self-reactive clones to bind both foreign and autoantigens. Both mechanisms can be involved in the pathogenesis of autoimmune disease.

The most striking observation in our study is the extensive idiotypic crossreactivity among $V_n$ J558+ autoantibodies with other autoantibodies and antibodies specific for foreign antigens. Idiotypes originally borne by RF, anti-DNA, and
### Table VII

**Specificity of Anti-Id mAbs Recognizing Crossreactive Idiotype on Antibodies Encoded by $V_H$ J558 Gene Family as Assessed by Competitive RIA**

| Idiotypic systems | Antigen specificity of idiotype | Binding of Id to corresponding antiidiotype* | Corresponding idiotype | Binding of labeled idiotype in presence of 500 ng of: |
|-------------------|---------------------------------|---------------------------------------------|------------------------|-----------------------------------------------|
|                   |                                 |                                             | UPC 10 (X24)\(\text{a}\) | J606 (J606) | MOPC460 (36-60) | J558 (J558) | TEPC15 (S107) | IDA 15 (QPC 52) | PY102 (7183) |
| IDA25-AIDA25.2    | IDA25 Id                         | 1.758 ± 169                                | 552 ± 28 (IDA25)\(\text{a}\) | 1,987 ± 25 | 1,790 ± 121 | 1,953 ± 111 | 1,848 ± 43 | 1,962 ± 42 | 1,764 ± 65 | 1,686 ± 64 |
| J558-CDS.2        | α1-3-Dextran                     | 4.291 ± 649                                | 1,018 ± 71 (MOPC 104) | 2,824 ± 197 | 3,284 ± 668 | 2,593 ± 65 | 840 ± 82 | 3,600 ± 24 | 3,355 ± 103 | 3,103 ± 73 |
| PY211-63-4        | HA of PR8                        | 18.545 ± 693                               | 6,900 ± 776 (MOPC 104) | 18,584 ± 211 | 15,589 ± 565 | 17,888 ± 661 | 11,957 ± 843 | 16,813 ± 574 | 18,405 ± 255 | 17,314 ± 661 |
| PY206-SN3A        | HA of X51                        | 12.376 ± 581                               | 1,885 ± 219 (PY206) | ND | ND | ND | ND | ND | ND |
| G5-HP20           | GAT                              | 10.923 ± 3,717                             | 2,700 ± 191 (G5) | 11,116 ± 718 | 9,541 ± 1,714 | 11,175 ± 718 | 11,275 ± 909 | 9,752 ± 724 | 9,825 ± 845 | 10,021 ± 414 |
| S6-65-ADH         | Ars                              | 17.808 ± 202                               | 3,837 ± 246 (36-65) | 17,909 ± 568 | 17,100 ± 751 | 16,866 ± 656 | 14,975 ± 568 | 16,738 ± 5,583 | 17,551 ± 584 | 16,581 ± 78 |

* Average cpm of triplicates ± SD.
\(\text{a}\) $V_H$ family.
\(\text{b}\) Corresponding idiotype used in competitive inhibition RIA.
anti-Sm antibodies were shared by a large fraction of our autoantibody panel. This result is expected, since Lymphert et al. (37) showed previously that there is a high idiotype connectivity among autoantibodies with various specificities obtained from newborn mice. However, our results clearly demonstrated that \( V_{\mu} \) J558-encoded autoantibodies share idiotypes with antibodies specific for foreign antigens that are also encoded by genes from the same family.

Taking into consideration our small panel of \( V_{\mu} \) J558+ autoantibodies and the small number of idiotypic systems used in this study, the frequency of idiotype crossreactivity is very high.

However, our data suggest that several pathways can be used for the activation of self-reactive clones. The activation of such clones requires complex immunologic mechanisms, including the activation of Th cells, the expression of Ia antigens on cell surfaces, and the possible exposure of intracellular antigen such as DNA or Sm. Data indicating that antibodies specific for foreign antigen can bind to self antigen (12, 13), combined with our data showing that “bona fide” autoantibodies can bind to foreign antigen suggest that autoreactive clones can be directly activated, in the case of T-independent antigens, or through Th cell cooperation, with T-dependent antigens.

This concept is in agreement with recent data showing that an intestinal infectious agent is responsible for the expansion of clones producing RF in 129/Sv mice (38). The RF synthesis was completely prevented in cesarian-derived and isolator-reared offsprings of RF+ dams.

Anti-Id antibodies are normally produced during an immune response elicited by conventional antigen (30, 40). Therefore, shared idiotopes between autoreactive antibodies and antibodies specific for foreign antigens can be targets for expansion of these autoreactive clones by anti-Id antibodies. Such a mechanism was envisioned by Plotz, who proposed that anti-Id antibodies against antiviral
antibodies can exert destructive effects by crossreactivity with cell-surface receptors (41).

In addition, the presence of IdX on autoantibodies with various specificities can explain the appearance of multiple autoantibodies during systemic autoimmune diseases such as systemic lupus erythematosus or rheumatoid arthritis, or during polyendocrine syndromes.

Studies in progress are aimed at investigating the activation of autoreactive clones by foreign antigens and anti-Id antibodies carrying the internal image of these foreign antigens in order to assess the pathologic significance of the polyspecificity among autoantibodies reported in this study.

Summary

We examined the binding to foreign antigens and the expression of crossreactive idiotypes by a panel of 20 murine monoclonal autoantibodies encoded by V genes from the V\textsubscript{H} J558 family. 9 of 20 antibodies bound to foreign antigens such as bacterial polysaccharides, poly(Glu\textsuperscript{50},Tyr\textsuperscript{50}), poly(Glu\textsuperscript{54},Lys\textsuperscript{37},Phe\textsuperscript{9}), arsonate, and lysozyme, known to interact with antibodies encoded by genes from the V\textsubscript{H} J558 family. A high proportion of our panel of autoantibodies expressed crossreactive idiotypes originally borne by monoclonal rheumatoid factors, anti-Sm, and anti-DNA antibodies, all encoded by V genes from the V\textsubscript{H} J558 family. Some of these V\textsubscript{H} J558\textsuperscript{+} autoantibodies shared crossreactive idiotypes with V\textsubscript{H} J558\textsuperscript{+} antibodies directed against foreign antigens such as influenza virus hemagglutinin, poly(Glu\textsuperscript{50},Ala\textsuperscript{50},Tyr\textsuperscript{10}), arsonate, and dextran.

The implications of these findings are discussed with respect to the process of activation of self-reactive clones.

Received for publication 13 March 1987 and in revised form 27 May 1987.

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