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Defective Self-Reactive Antibody Repertoire of Serum IgM in Patients with Hyper-IgM Syndrome

Sébastien Lacroix-Desmazes,* Igor Resnick,† Dorothea Stahl,* Luc Mouthon,* Teresa Espanol,‡ Jacov Levy,§ Srini V. Kaveri,* Luigi Notarangelo,¶ Martha Eibi,‖ Alain Fischer,⁎ and Michel D. Kazatchkine**

We have analyzed the self-reactive repertoires of IgM and IgG Abs in the serum of 19 patients with hyper-IgM syndrome (HIM) by means of a quantitative immunoblotting technique that allows for a quantitative comparison of Ab repertoires in health and disease by multiparametric statistical analysis. Normal tissue extracts of liver, lung, stomach, and kidney were used as sources of self Ags. Extracts of Pseudomonas aeruginosa and Staphylococcus epidermidis were used as sources of nonself Ags. We demonstrate a significant bias in repertoires of reactivities of IgM of patients with HIM with self Ags. Ab repertoires of IgM toward nonself Ags did not differ, however, between patients and controls. No difference was found between IgM repertoires of untreated patients and those of patients receiving substitutive treatment with i.v. IgG. IgG in the serum of HIM patients lacked reactivity with self Ags. No difference was found between IgM repertoires of untreated patients and healthy controls. In addition, little reactivity with self Ags of IgG in the serum of patients with HIM was detected. Our observations demonstrate that the selection of autoreactive B cells requires the presence of CD40-CD40L interactions essential for the selection of natural self-reactive B cell repertoires. The Journal of Immunology, 1999, 162: 5601–5608.

Immunodeficiency with hyper-IgM syndrome (HIM) is a rare disease characterized by normal or increased serum concentrations of IgM with decreased or absent IgG, IgA, and IgE (1, 2). HIM results from defective interactions between CD40 ligand (CD40L) on activated T cells and CD40 on B cells. The X-linked form of HIM (X-HIM) is characterized by defective CD40L due to deletions/insertions, point mutations, or truncation in the CD40L-encoding gene (3–12). Several in vivo and in vitro studies have documented that T cell-dependent isotype switch is strictly dependent on cognate interactions involving CD40 and CD40L and that impaired CD40-CD40L interactions inhibit the development of germinal centers and the generation of B memory cells (13–17). Patients with HIM lack germinal centers in secondary lymphoid organs (18). The patients suffer from recurrent upper and lower respiratory tract infections and also present with an unusual susceptibility to Pneumocystis carinii pneumonia and Cryptosporidium infection, suggesting impaired T cell functions. HIM patients often present with persistent neutropenia and may develop thrombocytopenia or other autoimmune manifestations such as hemolytic anemia and nephritis (1, 19). An increased incidence of autoantibodies, including anti-erythrocyte, anti-platelet, antithyroid, anti-nuclear, anti-cardiolipin, and anti-smooth muscle Abs, has been reported in patients with HIM (19–23).

Natural Abs of the IgM, IgG, and IgA isotypes that are reactive with a broad range of self Ags are present in normal serum (24–26). Ab repertoires of natural IgM and IgG toward self Ags are highly homogeneous among healthy individuals and remain invariant with aging (27–30). Evidence obtained in mice suggests that the selection of autoreactive B cells requires the presence of CD4+ T lymphocytes under conditions of both pathological and physiological autoimmunity (31–34).

In the present study, we analyzed the Ab repertoires of IgM and IgG in the serum of patients with HIM by means of a quantitative immunoblotting technique that allows for multiparametric statistical analysis of Ab reactivities with self and nonself Ags. We demonstrate that Ab repertoires of IgM toward self Ags are skewed in patients with HIM, whereas repertoires directed toward bacterial Ags do not differ between patients and healthy controls. In addition, little reactivity with self Ags of IgG in the serum of patients with HIM was detected. Our observations demonstrate that the lack of functional CD40-CD40L interactions and/or impaired T/B cell cooperation in HIM affect the selection processes of natural self-reactive B cell repertoires.

Patients and Methods

Patients

EDTA-plasma was obtained from 19 children (18 boys and 1 girl), between 3 and 14 years old, diagnosed with immunodeficiency with HIM. Twenty healthy young adult male blood donors with a mean age of 34 ± 5 years were used as normal controls. Previous studies from our laboratory had demonstrated that self-reactive Ab repertoires in serum remain highly homogeneous and invariant from early childhood to adulthood (29, 35).

Sixteen of the male patients had X-HIM. Two male patients presented with autosomal recessive HIM, and one female patient had secondary HIM associated with congenital rubella. Activated PBMC totally lacked expression of the CD40L Ag in 11 X-HIM patients, whereas expression of the Ag was low in 3 patients (2 patients with X-HIM and 1 patient with autosomal...
Sephacryl HR S-300 (Pharmacia, Uppsala, Sweden). The fraction of normal IgM (i.v. IgM) was obtained by submitting Pentaglobin doglobulin (a gift of the Central Laboratory of the Swiss Red Cross, Bern, l'Institut Pasteur, CIP A22) and 2-ME, 125 mM Tris-HCl (pH 6.8), containing 1.0 mM i.v. Ig demonstrated a dose-dependent decrease in the area under the curve corresponding to peaks of reactivity for concentrations of IgG between 50 and 400 μg/ml and of IgM between 5 and 50 μg/ml. Saturation was achieved for concentrations of IgG and IgM above 400 and 50 μg/ml, respectively. The reproducibility of the assay was 10% (variation coefficient). The 95% confidence interval of the mean area under the curve corresponding to each peak of immunoreactivity was 30% in the case of IgM and 25% in the case of IgG, as calculated by Student’s t test (27, 28).

Statistical analysis

Densitometric profiles of immunoblots were divided into sections corresponding to individual peaks of immunoreactivity. Respective peak areas were calculated in the case of each tissue extract. To discriminate between individual repertoires, peak areas corresponding to sections obtained with all self Ags were submitted simultaneously to principal component analysis (PCA) (38), using Mathematica (Wolfram Research, Champaign, IL) software. The repertoire of reactivities of each individual in a given sample was represented as a single symbol in a two-dimensional linear subspace. Discrimination between repertoires was assessed by submitting PCA data to linear discriminant analysis (LDA) and by subsequently comparing factors 1 of the LDA by Mann-Whitney U test. The statistical comparison required that factor 1 of the LDA accounted for >70% of the variance of the data. PCA of repertoires of Ab reactivities performed individually for each group of individuals further allowed the calculation of respective variances. Variances were compared by F test. We also quantitated total immunoreactivities of serum IgM with each source of Ags by computing the total area under the curves of the respective densitometric profiles. Mean values of total reactivities obtained with each source of Ag were compared between groups of individuals in a global analysis by Fisher’s test.

Analysis of IgM reactivity by ELISA

Ninety-six-well ELISA plates (Nunc, Roskilde, Denmark) were coated with human Ag H (a gift from Prof. J.-P. Carron, Institut National de la Sante et de la Recherche Medicale (INSERM), Unit 76, Paris, France), human laminin (Sigma), human thyroglobulin (Biogenesis, Poole, U.K.), human transferrin (Sigma), calf actin, and calf thymus DNA (Sigma) at 10 μg/ml in PBS, pH 7.4, and with human low density lipoprotein (LDL, a gift from J. Chevalier, INSERM, Unit 430, Paris, France) at 10 μg/ml in PBS, 2.7 mM EDTA, and 20 μM butylated hydroxytoluene (Sigma) overnight at 4°C. Plates coated with DNA had been pretreated with 10 μg/ml polyclonal anti-calf DNA IgG (Serotec, Oxford, UK). Plates were saturated with PBS containing 1% BSA (Sigma) in PBS for 30 min at 37°C. After a washing with PBS, the plates were incubated with decreasing concentrations of serum IgM to be tested for 1 h at 37°C before extensive washing with PBS and addition of goat anti-human IgM Abs coupled to alkaline phosphatase (Southern Biotechnology Associates). The background reactivity of serum IgM scored with nonsensitized plates, or with polyclonal DNA-coated wells in the case of DNA, was subtracted from the reactivity of IgM with the respective Ags. The mean values of the corrected IgM reactivities were compared between groups for each Ag by means of an one-sided Student t test.

Results

Reactivity with self Ags of serum IgM of patients with the hyper-IgM syndrome

The reactivity with self Ags of IgM in the serum of healthy blood donors and patients with HIM was analyzed by immunoblotting using extracts of normal homologous human kidney, lung, liver, and stomach as sources of self Ags. From 20 to 30 major peaks of reactivity were scored after blotting of IgM of healthy individuals with Ags in the extracts (Fig. 1 and data not shown). As previously reported (28, 35), the densitometric profiles of reactivity of IgM of healthy donors exhibited a strong homogeneity between individuals with regard to the nature of the protein bands recognized in all tissue extracts that were tested. From 15 to 20 major peaks of reactivity were also detected after blotting of IgM of patients with HIM with self Ags (Fig. 1 and data not shown). The densitometric profiles of IgM reactivities exhibited a strong homogeneity between patients with regard to the nature of the protein bands recognized and were also homogeneous with regard to the intensity of immunoreactivities in lung and stomach Ags, but not in kidney and liver Ags (Fig. 1 and data not shown).
To compare the densitometric profiles of reactivities of IgM of healthy donors and patients, we computed the arithmetic mean reactivity profiles with self Ags of IgM of the 20 healthy donors and 19 patients (Fig. 2). Most of the protein bands detected by IgM in the serum of healthy donors were also recognized by HIM IgM with lower intensity. Several of the IgM reactivities expressed by healthy subjects were not present in the repertoire of reactivities of IgM of HIM patients. The overall reactivity with self Ags of IgM of the HIM patients, as calculated by computing the mean area under the curve of the densitometric profiles obtained with each tissue extract, was significantly lower than that of healthy individuals in kidney, liver, and stomach Ags, but not in lung Ags (Table I).

To further compare the self-reactive repertoires of IgM of patients and healthy controls, we calculated the area of the peaks of immunoreactivity in densitometric profiles of IgM of each individual in each tissue extract. The data obtained with IgM of healthy donors were compared with those obtained in patients by PCA within a 31- to 56-dimension vector space, depending on the tissue extract, and fitted into the two-dimensional linear subspace that accounted for 55.6 to 69.4% of the variance and allowed the most powerful discrimination of the individuals (Fig. 2). The data obtained by PCA were then submitted to LDA. Factors 1 of LDA discriminated between repertoires of Ab reactivities with self Ags of healthy donors and patients with HIM (p < 0.001 in the case of all tissue extracts). PCA did not discriminate between self-reactive repertoires of HIM patients undergoing substitutive therapy with i.v. Ig and untreated patients (data not shown), indicating that the administration of normal IgG to HIM patients does not restore a physiological pattern of self-reactivity of IgM. PCA did not discriminate between self-reactive IgM Ab repertoires of patients with X-HIM and the patients with autosomal recessive HIM (data not shown). We then assessed the relative homogeneity of Ab repertoires of the 19 patients and 20 controls, by calculating the individual variances of the repertoires of self-reactivities in the case of each protein extract, by means of PCA. The variances did not differ significantly between healthy donors and HIM patients (Table II), demonstrating that self-reactive repertoires of IgM were homogeneous in both groups of individuals. Taken together, these data document that self-reactive repertoires of IgM of patients with HIM differ significantly from those of healthy donors.

We then analyzed the reactivity of IgM in the serum of healthy blood donors and patients with HIM in the case of several individual self Ags by ELISA. The reactivity of serum IgM of HIM patients with DNA and with protein autoantigens, i.e., actin, laminin, thyroglobulin, and transferrin, was significantly lower than that of healthy blood donors (Fig. 3). The reactivity of IgM from patients with HIM was, however, identical with that of healthy subjects in the case of Ag H and LDL (Fig. 3), suggesting that the nature of the autoantigen determines whether T cell help is required for the selection of autoreactive B cell clones.

Reactivity with nonself Ags of serum IgM of patients with the hyper-IgM syndrome

To investigate Ab repertoires against nonself Ags, we assessed the reactivity of IgM of patients with HIM with extracts of P. aeruginosa and S. epidermidis. The densitometric profiles of reactivity of IgM of healthy donors and HIM patients exhibited between 10 and 20 and between 5 and 10 major peaks of reactivity with the 2 sources of bacterial Ags, respectively (data not shown). The densitometric profiles of IgM of healthy donors exhibited a strong homogeneity between individuals with regard to the nature of the protein bands recognized and the intensity of the peaks, whereas densitometric profiles of IgM of patients were homogeneous in the case of P. aeruginosa extracts and heterogeneous in that of extracts of S. epidermidis (data not shown). The total reactivity of IgM of HIM patients with the bacterial extracts did not differ significantly from that of healthy individuals (Table III). We then computed the areas of the peaks of immunoreactivity in the densitometric profiles to allow for multiparametric analysis of the data. The data obtained in healthy donors and patients were compared by means of PCA within a 46- to 58-dimension vector space, depending on...
patients with HIM appeared to generate an IgM Ab repertoire to commensal bacteria similar to that of healthy individuals, contrasting with impaired Ab repertoires to self Ags.

**Reactivity with self and nonself Ags of IgG in serum of patients with the hyper-IgM syndrome**

We analyzed the repertoires of reactivity of IgG in the serum of 7 patients who had not been substituted with i.v. Ig and of 5 control healthy subjects. The concentration of IgG in patients’ serum was <0.4 mg/ml in 3 patients and ranged between 0.6 and 1.2 mg/ml in the 4 remaining patients. Reactivities of IgG were tested in serum diluted to achieve a final IgG concentration of 200 μg/ml. IgG of healthy donors strongly reacted with 20–30 protein bands, depending on the tissue extract (data not shown). As previously described (27), the patterns of reactivities were heterogeneous among healthy individuals with regard to both the nature of the protein bands recognized and the intensity of reactivities. No reactivity with self Ags and nonself Ags was seen with serum of the 3 patients who exhibited the lowest concentrations of serum IgG. IgG of the remaining 4 HIM patients exhibited weak reactivity with 10 to 15 protein bands in the kidney extract (data not shown). In contrast, a strong reactivity was observed with Ags in the *S. epidermidis* extract (data not shown). The total reactivity of IgG of HIM patients was significantly lower than that of healthy blood donors in the case of kidney Ags (*p*= 0.001), but not in that of *S. epidermidis* Ags (*p* = 0.66) (Fig. 5 and Table V), indicating a selective bias in the repertoire of reactivities of IgG toward self Ags.

The data obtained upon immunoblotting of IgG were compared by means of PCA within 44- and 42-dimension vector spaces, in the case of kidney and *S. epidermidis* extracts, respectively, and fitted into 2-dimensional linear subspaces that accounted for 91.4 and 70.0% of the variance, respectively. PCA discriminated between healthy donors and patients in the case of kidney Ags (*p* <

### Table I. Total reactivity with self-Ags of IgM in the serum of healthy individuals and patients with hyper-IgM syndrome

<table>
<thead>
<tr>
<th>Tissue Extract</th>
<th>Healthy donors</th>
<th>HIM patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>41.7 ± 5.0</td>
<td>28.3 ± 7.6</td>
</tr>
<tr>
<td>Lung</td>
<td>48.3 ± 9.7</td>
<td>41.8 ± 9.0</td>
</tr>
<tr>
<td>Liver</td>
<td>39.1 ± 8.2</td>
<td>33.1 ± 10.9</td>
</tr>
<tr>
<td>Stomach</td>
<td>37.3 ± 6.2</td>
<td>25.1 ± 5.5</td>
</tr>
</tbody>
</table>

*Total areas under the curves of densitometric profiles of immunoreactivity of IgM with self-Ags in extracts of kidney, lung, liver, and stomach were calculated. Results are the mean ± SD of the total reactivity within each group of individuals. Total reactivity is expressed as arbitrary units. Significant differences are indicated, as assessed by Fisher’s test (*†, p < 0.05; †, p < 0.001*).

### Table II. Total variances of the self-reactive repertoires of IgM of healthy individuals and of patients with hyper-IgM syndrome

<table>
<thead>
<tr>
<th>Tissue Extract</th>
<th>Total Variance of Repertoires of Reactivity of IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors</td>
<td>HIM patients</td>
</tr>
<tr>
<td>Kidney</td>
<td>1071.6</td>
</tr>
<tr>
<td>Lung</td>
<td>2927.2</td>
</tr>
<tr>
<td>Liver</td>
<td>1768.7</td>
</tr>
<tr>
<td>Stomach</td>
<td>2806.4</td>
</tr>
</tbody>
</table>

*Total variances of repertoires of reactivities of IgM in whole serum with self-Ags in extracts of kidney, lung, liver, and stomach were calculated separately in a 31- to 56-dimension vector space, depending on the tissue extract. Differences were not significant, as assessed by F test.*
Discussion

In the present study, we have characterized the Ab repertoires of IgM and IgG in the serum of patients with the hyper-IgM syndrome. We demonstrate a significant bias in repertoires of reactivities of IgM of patients with HIM with self Ags. Furthermore, IgG of HIM patients lacked reactivity with self Ags, in contrast with IgG of healthy controls, when tested at similar concentrations. Repertoires of reactivities of HIM IgM with foreign Ags did not differ from those of IgM in the serum of healthy individuals. These results indicate that the lack of functional CD40-CD40L interactions or defective T cell/B cell cooperation impact on the selection of physiological self-reactive Ab repertoires.

Natural autoreactive IgM and IgG Abs are found in the serum of healthy individuals of young adults and between males and females with regard to reactivity with self Ags (29, 35, 40). Significant alterations in self-reactive Ab repertoires have been observed in autoimmune conditions, such as myasthenia gravis and systemic lupus erythematosus (41, 42).

Here we investigated Ab repertoires in the serum of 19 patients with HIM, 16 of whom had X-HIM. We observed that the densitometric patterns of reactivity with self Ags of IgM in the serum of patients with HIM were homogeneous among the patients but differed significantly from those of healthy donors. Several peaks of self-reactivity of IgM in normal serum were lacking in the serum of patients and controls.

Table III. Total reactivity with foreign Ags of IgM in the serum of healthy individuals and patients with hyper-IgM syndrome

<table>
<thead>
<tr>
<th>Tissue Extract</th>
<th>Healthy donors</th>
<th>HIM patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>36.7 ± 6.3</td>
<td>32.5 ± 9.7</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>32.8 ± 7.7</td>
<td>28.3 ± 11.5</td>
</tr>
</tbody>
</table>

Total areas under the curves of densitometric profiles of immunoreactivity of IgM with Ags in extracts of P. aeruginosa and S. epidermidis were calculated. Results are the mean ± SD of the total reactivity within each group of individuals. Total reactivity is expressed as arbitrary units. Differences were not significant, as assessed by Fisher’s test.

Table IV. Total variances of the repertoires of reactivity of IgM of healthy subjects and of patients with hyper-IgM syndrome with Ags in bacterial extracts

<table>
<thead>
<tr>
<th>Tissue Extract</th>
<th>Healthy donors</th>
<th>HIM patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. aeruginosa</td>
<td>54.6</td>
<td>64.5</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>64.5</td>
<td>130.6</td>
</tr>
</tbody>
</table>

Total variances of the repertoires of reactivity of IgM in whole serum with extracts of P. aeruginosa and S. epidermidis were calculated by separate PCA of the data corresponding to each group. The data were analyzed in a 46- to 58-dimensional vector space, depending on the bacterial extract. Differences were not significant, as assessed by F test.
of HIM patients. Principal component analysis discriminated between self-reactive IgM repertoires of patients and healthy donors. The variances of individual Ab repertoires were of a similar order of magnitude in both groups, indicating that healthy donors and patients with HIM represent homogenous groups of individuals characterized by distinct patterns of recognition of self Ags. These observations emphasize the key role of T lymphocytes for establishment of natural self-reactive repertoires of IgM Abs, as suggested by previous studies in mice. Thus, it has been shown that euthymic and athymic (nu/nu) BALB/c mice present with distinct self-reactive repertoires of IgM (34). The transfer of syngeneic T cells to athymic mice restored high frequencies of autoreactive precursor B cell clones (33) and altered repertoires of reactivities of natural autoantibodies, with patterns almost identical with those of euthymic mice (34). The transfer of syngeneic T cells to nude mice was also shown to restore normal titers of serum IgG (33, 34).

A number of studies document a role for IgG in the selection of IgM repertoires. Thus, maternal IgG decreases the concentration and alters the pattern of reactivity of serum IgM in newborn mice (34, 43). Treatment with i.v. IgG of autoimmune patients, is followed by altered titers of specific IgM and IgG autoantibodies (44–46). In patients with HIM, the administration of i.v. Ig often results in a dramatic and long-lasting decrease of serum IgM levels (21, 23, 47–49). The latter decrease may reflect the effects of i.v. Ig on B cell repertoires or, alternatively, be a consequence of a better control of recurrent infections. In our study, however, no difference in the serum concentration of IgM was observed between patients substituted with i.v. Ig and untreated patients (1). In addition, PCA did not discriminate between self-reactive Ab repertoires of IgM of i.v. Ig-treated and untreated HIM patients. Since the concentration of IgG in the serum of the 12 patients treated with i.v. Ig was significantly lower than in the serum of healthy individuals (2.6 ± 2.1 mg/ml), the possibility remains that IgG was at too low a concentration in plasma to efficiently influence the selection of B cell repertoires. Thus, it is unclear at present whether alterations in the self-reactive repertoires of IgM in patients with HIM are strictly dependent on defective T cell/B cell signaling or whether these alterations are also a consequence of decreased levels of autologous IgG.

Increased concentrations of IgM in the serum of patients with HIM were not associated with an increase in the overall reactivity of IgM with self Ags. In fact, the total autoreactivity of IgM of patients was significantly lower than that of controls in the case of three of the four tissue extracts that we tested as sources for self Ags. Our results agree with a previous report on HIM patients who were devoid of anti-Gal1-3Gal Abs as compared with healthy individuals (50). However, in our study, the bias in IgM autoreactivity was restricted to protein Ags, suggesting that the role of T cell help in the selection of self-reactive B cell clones depends on the nature of the autoantigens. It may be speculated that autoimmune manifestations which were reported to occur in patients with HIM patients. Principal component analysis discriminated between self-reactive IgM repertoires of patients and healthy donors. The variances of individual Ab repertoires were of a similar order of magnitude in both groups, indicating that healthy donors and patients with HIM represent homogenous groups of individuals characterized by distinct patterns of recognition of self Ags. These observations emphasize the key role of T lymphocytes for establishment of natural self-reactive repertoires of IgM Abs, as suggested by previous studies in mice. Thus, it has been shown that euthymic and athymic (nu/nu) BALB/c mice present with distinct self-reactive repertoires of IgM (34). The transfer of syngeneic T cells to athymic mice restored high frequencies of autoreactive precursor B cell clones (33) and altered repertoires of reactivities of natural autoantibodies, with patterns almost identical with those of euthymic mice (34). The transfer of syngeneic T cells to nude mice was also shown to restore normal titers of serum IgG (33, 34).

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HIM (2, 19) may be dependent on an impaired control of autoreactivity, secondary to defective T cell-B cell interactions. Increased titers of IgM in patients with HIM have been suggested to reflect a compensatory mechanism in individuals confronted with chronic stimulation of the immune system by infectious agents (1, 2). If that were the case, one would have expected a bias in the repertoire of reactivities of IgM toward bacterial Ags. However, we did not observe qualitative or quantitative differences in the repertoires of IgM toward Ags of P. aeruginosa and S. epidermidis. These observations suggest that IgM immune responses against foreign Ags similar to those of healthy individuals may occur in the absence of normal autologous T cells. In this respect, it is noteworthy that a proportion of B cells in patients with X-HIM may be induced to undergo somatic mutation (12).

There was almost no reactivity toward self Ags of IgG in the serum of the seven untreated patients with X-HIM whom we tested. In contrast, when tested at a similar concentration, IgG in the serum of healthy individuals reacted with several protein Ags in homologous tissue extracts. The lack of self reactivity of IgG in HIM serum may be a consequence of defective signaling through CD40 and CD40L, suggesting the requirement for normal T and B cell interactions for the generation of natural autoantibodies of the IgG isotype. An alternative hypothesis is that IgG autoantibodies are produced in HIM but that IgG autoreactivity is masked in serum (51, 52).

It is noteworthy that a proportion of B cells in patients with X-HIM may be induced to undergo somatic mutation (12). Taken together, our observations emphasize the key role of T lymphocytes in establishing natural self-reactive Ab repertoires.

Acknowledgments

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