THE AUTOANTIGEN-BINDING B CELL REPERTOIRES OF NORMAL AND OF CHRONICALLY GRAFT-VERSUS-HOST-DISEASED MICE

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Antibodies binding to a variety of self antigens have been found in the sera of normal individuals (1). Autoantibodies have been identified that bind to nuclear antigens (DNA, RNA, histones), to cellular antigens, to cell-surface membrane molecules, and to serum proteins (2-6). Cells producing such autoantibodies have been cloned as murine hybridomas from fetal, newborn, and adult normal mice (2-4, 7) and as EBV-transformed B cells or as hybridomas from normal human individuals (4, 5, 8, 9). These findings have implied that the normal repertoire of the immune system includes self antigen-binding B cells.

Autoantibodies binding to self antigen have also been found in a variety of autoimmune disorders, among them chronic GVHD (reviewed in reference 10). This disease in mice closely resembles SLE in man. Autoantibodies to nuclear antigens (ANA),1 to thymocytes, and to erythrocytes are found in the serum. Immune complex glomerulonephritis (ICGN) develops in later stages of the disease (10, 11). Chronic GVHD can be induced in normal, not genetically autoimmune disease-prone F1 mice by injection of Th cells of one parent, i.e., of alloreactive cells (10, 12-14).

Qualitatively, therefore, the reactivity pattern of B cells appears to be similar in normal and in GVHD mice in that it contains autoantibody-producing cells. In this paper we present a quantitative analysis of the frequencies of autoantibody-producing B cells in GVHD and in normal mice. We generate collections of hybridomas that allow the production of sufficient quantities of secreted antibody needed for these analyses. B cells have to be activated before they will fuse with the Sp 2/0 line to generate hybridomas (15). We use a variety of procedures to activate splenic B cells in vivo and in vitro. We activate (C57BL/6 X DBA/2)F1 B cells in vivo by injection of alloreactive Th cells of one parent (GVHD), by a foreign antigen in a secondary response (SRBC), or by LPS as a polyclonal activator. We also use B cells in experimentally unstimulated animals that are activated by the normal background. Finally we activate B cells in vitro by LPS...
and by alloreactive T cells. The quality of the response is monitored by the fine specificity and the class of antibody produced.

Materials and Methods

Mice. Female (C57BL/6J X DBA/2)F1 and DBA/2 mice, as well as Lewis rats, 6–10 wk old, were obtained from the Institut für Biologisch-Medizinische Forschung, AG., Füllinsdorf, Switzerland.

Immunizations. Graft-vs.-host reaction (GVHR) was induced in female (C57BL/6J X DBA/2)F1 mice by intravenous injection of 2 x 10^7 nylon wool–purified splenic T cells (16) of female DBA/2 mice. Fusion of the spleen cells of individual mice was performed at different times after the induction of the GVHR as indicated in the results. In vivo activation of spleen cells with LPS was performed by injecting (C57BL/6J X DBA/2)F1 mice intravenously with 100 µg LPS (S-form of Salmonella abortus equi, a kind gift of Drs. C. Galanos and O. Lüderitz, Max Planck Institut für Immunbiologie, Freiburg, Federal Republic of Germany). Fusions were performed 3 days after the injection of LPS. A secondary SRBC response was induced in (C57BL/6J X DBA/2)F1 mice by two intravenous injections of 10^8 SRBC on day 0 and day 14. Fusion of the spleen cells of these mice was done on day 19.

Cell Cultures. Stimulation of spleen cells in vitro was done in several ways. (C57BL/6J X DBA/2)F1 spleen cells at a concentration of 10^6 cells/ml, were stimulated by coculture with 2 x 10^5 irradiated (1,500 rad) alloreactive C57BL/6 anti–BALB/c Th cells per milliliter. Fusion was performed at day 3 of culture. LPS stimulation of (C57BL/6J X DBA/2)F1 spleen cells in vitro was performed at a concentration of 5 x 10^5 spleen cells per milliliter in the presence of 50 µg/ml of LPS. Fusion was performed at day 5 of culture. (C57BL/6J X DBA/2)F1 fetal liver cells taken at day 17 of gestation were stimulated at a concentration of 10^8 cells per milliliter with 50 µg/ml of LPS. Fusion was performed at day 7 of culture. All above-mentioned cultures were performed in serum-substituted culture medium (17).

Fibroblasts Ltk- used for indirect immunofluorescence were grown overnight onto eight-hole microscope slides (Cell-Line Associates, Newfield, NY) in RPMI 1640 medium containing 10% FCS.

Production of mAbs. In vivo or in vitro–stimulated (C57BL/6J X DBA/2)F1 spleen or fetal liver cells were fused with the nonsecreting, azaguanine-resistant hybridoma cell line SP 2/0 according to the original protocol of Kohler and Milstein (18). The fused cells were plated into microtiter plates under limiting dilution conditions in the presence of rat thymocytes (3 x 10^4/ml) as filler cells (19). Autoantibody production was measured from those cultures that showed positive growth and, according to Poisson’s distribution, had a >98% probability of being derived from a single fusion event, i.e., a hybridoma clone.

Assays. The number of IgM- and IgG-secreting cells was determined using the protein A plaque assay as originally described by Gronowicz et al. (20). The presence of autoantibodies to thymocytes and nuclear antigens in the serum was determined as described (12–14). Whether mice had ICGN was determined with direct immunofluorescence as previously described (11). Cryostat sections (4 µm) of mouse kidney, liver, stomach, lung, and intestine, as well as human kidney, liver, esophagus, and small intestine used for indirect immunofluorescence tests were fixed in acetone for 10 min and then air dried. Fibroblasts used for indirect immunofluorescence were fixed for 15 min in ethanol containing 5% acetic acid at −20°C. Cells were rehydrated in PBS.

Indirect immunofluorescence on sections or fibroblasts was done by incubating these for 30 min with hybridoma culture supernatant. Thereafter they were washed with PBS for 30 min and then incubated with FITC-labeled goat anti–mouse IgM + IgG (Jackson Research Laboratory, Avondale, PA) diluted 1:20 in PBS. After extensive washing with PBS the slides were read in an Orthoplan microscope (E. Leitz, Wetzlar, Federal Republic of Germany) with incident illumination.

Anti-DNA antibodies were determined as described by Smecsek (21). Briefly, Elisa plates were precoated with a 0.05% (W/V) solution of protamin sulfate and then coated
overnight at 4°C with native salmon sperm DNA at a concentration of 6 μg/ml. Hybridoma culture supernatants to be tested for binding were diluted 1 in 1 with PBS containing 0.1% Tween-20 and 2% BSA. A one in 1000 diluted alkaline phosphatase labelled goat anti-mouse IgM + IgG (Jackson Research Laboratory) was used as conjugate. Binding was assayed by the presence of enzymatic activity using p-nitrophenylphosphate as a substrate.

Ig Class and Subclass Determination. The Ig (sub)classes produced by the hybridomas were determined in an ELISA. ELISA plates (Dynatech, Plochingen, Federal Republic of Germany) were coated with 10 μg/ml affinity-purified goat anti-mouse IgM + IgG (Jackson Immuno Research, Avondale, PA) for 2 h at room temperature. After extensive washing of the plates with PBS, 50 μl of culture supernatant, diluted 1:1 in PBS containing 0.2% Tween 20, was applied to the wells for a period of 2 h. Thereafter, the plates were washed five times with PBS and incubated with 50 μl of 1:500 diluted (PBS + 0.2% Tween 20) alkaline phosphatase–labeled goat anti-mouse IgG, IgM, IgG1, IgG2a, IgG2b, IgG3 (κ or λ) (all purchased from Southern Biotechnology Associates, Inc., Birmingham, AL), for 2 h. After extensive washing with PBS, 200 μl of the substrate p-nitrophenyl phosphate (1 mg/ml) was added. After a 30-min incubation period the plates were read in a Titertek ELISA reader (Flow Laboratories, Rickmansworth, United Kingdom) at 405 nm.

Results

In Vivo Activation and Autoimmune Response of (C57BL/6J × DBA/2)F1 Splenic B Cells by Injection of DBA/2 T Cells. It is known that injection of DBA/2 T cells into adult (C57BL/6J × DBA/2)F1 mice can lead to chronic GVHD that closely resembles SLE (10). Therefore, we monitored in such mice the total activation of the B cell compartment in the spleen to Ig-secreting cells, the formation of ANAs and autoantibodies to thymocytes, and the development of ICGN with time after injection of the T cells. Normal mice that were not injected with T cells were used as controls. The results summarized in Table I show activation to IgM- and IgG-secreting cells within a maximum of 2 wk after T cell injection. Numbers of IgM-secreting cells returned to normal levels at 5–8 wk after T cell injection.

The levels of IgG-secreting cells at 2 wk after T cell injection are 10 times higher than those of IgM-secreting cells and 50 times higher than the background in unstimulated mice. Furthermore, they remain elevated at least five times above the normal background even 8 wk after the T cell injection. The mice have autoantibodies against thymocytes in their serum as early as 1 wk after T cell injection and up to 8 wk afterward. ANAs could not be detected at 1 wk, but later on they were detected throughout.

Mice at various times after T cell injection served as a source for activated spleen cells, which were used in fusions with the azaguanine-resistant SP 2/0 hybridoma cell line to develop repertoires of hybridomas. One mouse used for fusion at 5 wk after T cell injection and the two mice used for fusion at 8 wk had developed ICGN. This is a sign that the mice used in the present study indeed developed SLE-like GVHD.

Frequencies of Autoantibody-secreting Hybridomas Obtained from Spleen Cells of (C57BL/6J × DBA/2)F1 Mice Stimulated in Various Ways. Repertoires of hybridomas were prepared by fusing spleen cells from (C57BL/6J × DBA/2)F1 mice activated in different ways. In vivo they were stimulated by parental DBA/2 T cells, i.e., by alloreactive cells, which could induce GVHD. Spleen cells were fused before as well as after the manifestation of the disease, i.e., at 1, 2, 5, and
8 wk. Other mice were stimulated in vivo with the polyclonal activator LPS, or in a secondary response with the antigen SRBC. In vitro spleen cells were stimulated either with alloreactive Th cells, or with LPS. Finally, hybridoma repertoires were also made from unstimulated adult spleen cells, and from fetal liver cells stimulated in vitro with LPS.

Successful fusion events were plated under conditions of limiting dilution. Only those hybridomas were collected and screened that, according to Poisson's distribution, had a >98% probability of being derived from a single fusion event.

Frequencies for successful fusion events differed for the spleen cell preparations from mice activated in different ways. It was one in $2 \times 10^7$ for normal spleen cells, one in $5 \times 10^5$ for mice injected with either SRBC or with alloreactive T cells (GVHD mice) and one in $2 \times 10^5$ for mice injected with LPS. This reflects the total status of activation of the B cell compartments in spleen and suggests, as previously shown with in vitro- and in vivo-activated spleen cells (15), that activated but not resting cells are the main fusion partners in the generation of hybridomas.

The antibodies produced by these hybridoma collections were screened for their capacity to bind to (C57BL/6J × DBA/2)F1 kidney cryosections or to DNA. Table II summarizes these analyses. Hybridomas producing autoantibodies were found in all groups. Their frequencies among all hybridomas ranged from 7.1% (GVHD at 2 wk) to 17.1% (LPS in vivo); within the GVHD mice this frequency varied from 7.1% to 11.6%. None of the 47 anti-SRBC mAbs crossreacted with the tested self antigens. Data in Table II indicate that the frequencies of hybridomas producing autoantibodies binding to kidney or to DNA are not

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### Table I

**Activation and Autoimmune Reactions of Splenic B Cells of (C57BL/6J × DBA/2)F1 Mice Injected with DBA/2 Splenic T Cells**

| Animal number | Time after injection of T cells | Ig-producing cells per 10^6 spleen cells at time of fusion | Autoantibodies in serum at time of fusion | ICN$^\dagger$ |
|---------------|--------------------------------|----------------------------------------------------------|------------------------------------------|-----------|
|               |                                | IgM | IgG | ANA | Antithymocyte | |
| 1$^\dagger$   | 0                              | 420 | 397 | -   | -             | -         |
| 2$^\dagger$   | 0                              | 390 | 500 | -   | -             | -         |
| 3$^\dagger$   | 0                              | 380 | 520 | -   | -             | -         |
| 4$^\dagger$   | 1                              | 860 | 3400 | -   | +             | -         |
| 5$^\dagger$   | 1                              | 850 | 3660 | -   | -             | -         |
| 6$^\dagger$   | 2                              | 2910 | 24930 | ++ | ++ | -         |
| 7$^\dagger$   | 2                              | 2300 | 23080 | ++ | ++ | -         |
| 8$^\dagger$   | 5                              | 350 | 2300 | ++ | ++ | -         |
| 9$^\dagger$   | 5                              | 370 | 4780 | ++ | ++ | -         |
| 10$^\dagger$  | 5                              | 370 | 13010 | ++ | ++ | +         |
| 11$^\dagger$  | 8                              | 400 | 4830 | ++ | ++ | +         |
| 12$^\dagger$  | 8                              | 230 | 2260 | ++ | ++ | ++        |

* At these various times spleen cells of these mice were prepared and fused with the Sp2/0 line to obtain the hybridomas described in the following results.

† $\dagger$, detectable in assays described in the Materials and Methods; $\ddagger$, not detectable.

$^\dagger$ Normal mice not injected with T cells were used as controls.
Table II
Frequencies of Autoantibody-producing B Cell Hybridomas Derived in Fusion with Unstimulated Fetal Liver and Spleen Cells, and with Spleen Cells Stimulated by Various In Vivo and In Vitro Procedures

| Group                      | Number of hybridomas | Number of hybridomas producing tested autoantibodies | Percentage |
|---------------------------|----------------------|-----------------------------------------------------|------------|
| Unstimulated spleen cells | 112                  | 18                                                  | 16.1       |
| LPS-stimulated fetal liver cells | 236                  | 28                                                  | 11.9       |
| SRBC                      | 213†                 | 23                                                  | 10.9       |
| T cell induced in vitro   | 184                  | 16                                                  | 8.7        |
| LPS in vivo               | 181                  | 31                                                  | 17.1       |
| LPS in vitro              | 324                  | 22†                                                 | 6.8†       |
| GVH (1 wk)                | 22                   | 2                                                   | 9.1        |
| GVH (2 wk)                | 511                  | 22                                                  | 7.1        |
| GVH (5 wk)                | 129                  | 15                                                  | 11.6       |
| GVH (8 wk)                | 122                  | 11                                                  | 9.0        |

* For details see Materials and Methods and text. Within one group several mice were fused in individual fusion experiments. Since no differences could be found in the repertoires of these individual mice, data in the table are pooled from several fusion experiments.
† Number of hybridomas producing autoantibodies that bind to kidney section and/or DNA.
§ 47 of these hybridomas produced SRBC-specific antibodies and none of them crossreacted with the self antigens tested.
∥ DNA binding was not tested in this group.

Significantly different between those derived from GVHD mice and those from mice of the same strain activated in different ways. Moreover, the frequencies of hybridomas producing these autoantibodies does not change with time after injection of the T cells. This suggests that the development of ICGN at later times after induction is not simply due to an increased number of activated splenic B cells producing these autoantibodies.

Different Cellular Specificities of Autoantibodies Detected by Binding to Kidney Sections. IgG produced by the hybridomas were initially screened for their capacity to bind to kidney cryosections. The ones found positive were then screened for binding to different organs of mice and humans, and to murine fibroblasts. Different staining patterns were found that allowed us to form five groups of autoantibody-producing hybridomas according to the binding of the antibodies to different cell structures (Fig. 1). A strong staining of the brush border of the proximal tubules of kidneys was seen with antibodies that were restricted to hybridomas obtained by fusion with GVHD spleen cells, whereas other organs and fibroblasts were not stained. Antibodies that stained a web in the cytoplasm of fibroblasts in a fine or coarse network-like pattern could be directed against cytoskeleton proteins. In frozen sections we observed a staining of connective tissue cells, like kidney, mesangial cells, and sometimes of smooth muscle cells and nerve sheaths. In addition, a few antibodies showed a bright staining of epithelial cells, e.g., squamous cells, but staining was virtually negative on connective tissue cells and fibroblasts. They could be directed against keratin or prekeratin proteins. In all groups, antibodies could be found that stained
FIGURE 1. Indirect immunofluorescence test on frozen sections of murine kidneys, stomach and on cultured fibroblasts. (A) Autoantibody directed against the brush border area of the proximal tubuli of the kidney. × 680. (B) Autoantibody directed against mitochondria. Granular staining of the cytoplasm of kidney tubuli. × 680. (C) Autoantibody directed against cytoskeleton tested on cultured fibroblasts. × 855. (D) Autoantibody directed against smooth muscle cells tested on a stomach wall. × 210.
smooth muscle cells of vessels, intestine, stomach, and esophagus, as well as antibodies that appeared directed against nuclei and/or nucleoli. Six antibodies showed a strong web-like staining of all cells and, in particular, of their nuclei and/or nucleoli.

Antibodies grouped in the antimitochondrial pattern stained predominantly total epithelial cells of kidney tubules in a granular pattern and, to a lesser extent, liver and stomach epithelial cells.

A summary of these results is given in Table III. As shown, autoantibodies to cytoskeletal structures are most predominant in almost all groups, being most frequent in hybridoma collections of fetal liver cells and of normal spleen cells. We do not consider the differences in frequencies of specificities in the different hybridoma collections to the different self antigens to be significant. On the other hand, autoantibodies with specificities for proximal tubular brush border were only found within the set of GVHD-derived hybridoma. Six GVHD mice produced such antibodies. It remains to be determined with a wider spectrum of autoantigens, whether other autoantigens exist to which autoantibodies are only made in GVHD mice.

A majority, i.e., 22 out of 28 DNA binding antibodies, were found also to bind to other cell structures (Table IV). 11 of these antibodies bind to nuclei, and another 11 to cytoskeletal structures.

Ig (Sub)classes of the Monoclonal Autoantibodies. The class or subclass of Ig with binding specificities for autoantigens derived from the differently activated B cell populations was determined by an ELISA described in the Materials and Methods section. In fact, included in this class and subclass determination were the Ig molecules produced by all the hybridomas of the differently activated B cells, regardless of whether they were found to be binding to a self antigen or not.

Practically all of the monoclonal autoantibodies were derived from nonautoimmun-
TABLE IV

DNA-binding Monoclonal Autoantibodies and their Crossreactivity with Different Cellular Structures

| Group*                             | Number of hybridomas | Number of DNA-binding monoclonal autoantibodies | Immunofluorescence of autoantibodies with DNA binding capacity |
|------------------------------------|----------------------|-------------------------------------------------|---------------------------------------------------------------|
|                                    |                      |                                                 | Nuclei | Cytoskeleton | No immunofluorescence |
| Unstimulated spleen cells          | 60                   | 1                                               | 1      | 1            | -                 |
| LPS-stimulated fetal liver cells   | 236                  | 1                                               | 1      | -            | -                 |
| SRBC in vivo                       | 211                  | 1                                               | -      | 1            | -                 |
| T cell induced in vitro            | 184                  | 7                                               | 1      | 4            | 2                 |
| LPS in vivo                        | 181                  | 4                                               | 1      | -            | 3                 |
| GVH (1 wk)                         | 22                   | -                                               | -      | -            | -                 |
| GVH (2 wk)                         | 311                  | 10                                              | 6      | 3            | 1                 |
| GVH (5 wk)                         | 129                  | 2                                               | 1      | 1            | -                 |
| GVH (8 wk)                         | 75                   | 2                                               | -      | 2            | -                 |

* For details see Material and Methods.

TABLE V

IgM/IgG Class Distribution in Hybridomas Derived from Spleen Cells Activated by Different Procedures and from Fetal Liver

| Group                            | Total hybridomas producing: | Autoantibody-secreting hybridomas producing: |
|----------------------------------|-----------------------------|---------------------------------------------|
|                                  | IgM | IgG | IgM | IgG |
| Unstimulated spleen cells        | 69.6 | 30.4 | 100 | 0   |
| LPS-stimulated fetal liver cells | 88.5 | 11.5 | 100 | 0   |
| SRBC in vivo                     | 51.2 | 48.8 | 100 | 0   |
| T cell induced in vitro          | 98.5 | 1.5  | 100 | 0   |
| LPS in vivo                      | 89.4 | 10.6 | 100 | 0   |
| LPS in vitro                     | 99.2 | 0.8  | 100 | 0   |
| GVH (1 wk)                       | 36.4 | 63.6 | 0   | 100 |
| GVH (2 wk)                       | 10.3 | 89.7 | 9.1 | 90.9 |
| GVH (5 wk)                       | 24.0 | 76.0 | 15.3| 86.7 |
| GVH (8 wk)                       | 58.2 | 41.8 | 18.2| 81.8 |

mune mice, i.e., F1 mice not injected with parental T cells are of the IgM isotype (Table V). This finding is not surprising for hybridomas made with polyclonally stimulated B cells (LPS- and Th cell–induced), since >90% of these hybridomas produce IgM antibodies (Table V).

However, 30% of the hybridomas derived from normal mice and 49% (42% after subtraction of hybridomas producing anti-SRBC antibodies) of the hybridomas of mice twice injected with SRBC produce IgG antibodies. Nevertheless, none of these IgG monoclonal antibodies bound to kidney cryosections, nor to DNA (Table V). This indicates that under normal conditions autoantigen-binding B cells can only be stimulated to IgM, but not to IgG secretion.

In marked contrast, many (between 42%–90%) of the GVHD-derived hybri-
domas produce IgG antibodies and considerably fewer IgM. The relative frequencies of IgM- and IgG-producing hybridomas changed with time after injection of the parental T cells, i.e., with propagation of the GVHD-effect (Table V), in agreement with the differently changing levels of IgM- and IgG-secreting cells found in the spleen (Table 1). Furthermore, as shown in Table V, 80-90% of the monoclonal autoantibodies are of the IgG class and only 10-20% are of the IgM class. The γ2a isotype is most predominantly used (46%) in the sum of all GVHD hybridomas, followed by γ1 (29%) and γ2b (12%) (Table VI). The γ3 isotype appears to be rare within autoantibodies of the hybridoma collections derived from the GVHD mice. All but one of the GVHD-derived monoclonal autoantibodies use κ light chains.

Discussion

For an evaluation of the repertoires of B cells that we have investigated in this paper, it is important to realize that these repertoires represent those B cells that were in an activated state at the time of fusion. Earlier work (15) has shown that activated B cells can be fused to form hybridomas at least 100 times more successfully than resting B cells. With the assumption that every activated B cell, at a given stage of activation, fuses equally successfully regardless of its antigen specificity, we conclude that the hybridomas generated in these fusions, in all likelihood, represent an average sampling of the activated B cell repertoire of the IgM- as well as the IgG-producing cells. Furthermore, since we have plated the originally successful fusion events at limiting dilutions, we have even excluded a possible selection of the best-growing hybridomas from cultures with more than one fusion event.

The transfer of Th cells of one parent into an F1 host which eventually leads to chronic GVHD and to SLE-like disease, results in a strong activation of the B cell compartment of the host. In fact, the strength of activation by these alloreactive Th cells appears comparable to the activation by LPS injected in vivo. Since alloreactive T cells are potential activators of all MHC class II-expressing B cells in the F1 host regardless of their antigen specificity, it can not be excluded that this strong activation is, at least to a large part, polyclonal. Activation of the B cell compartment during a secondary response to the foreign antigen SRBC is also very strong. Only one-quarter of the B cell response appears
SRBC specific. The repertoire of binding specificities of B cells activated by allogeneic T cells in vivo is largely indistinguishable from that activated by LPS in vivo, as well as in vitro, and is also indistinguishable from the three-quarters of the B cell response to SRBC that appears not to be SRBC specific. This supports the notion that the early in vivo activation by allogeneic T cells, as well as a large part of the SRBC-induced response, is polyclonal.

A transient polyclonal activation of IgM-producing cells during the first period of the GVHR has been described (22, 23). The kinetics of this polyclonal activation are similar to the kinetics of the total IgM-producing B cell population in GVHR (23). In spite of the strong increase in IgG production, no IgG PFCs or IgG antibodies to non-self antigens were previously found in F1 with GVHD (23, 24). From these findings it was concluded that the autoantibody formation in GVHD F1 mice is due to the specific triggering of a certain population of autoreactive B cells and not to a polyclonal B cell stimulation. The data presented here appear to argue against such specific triggering of autoreactive B cells, since the frequencies of autoreactive B cells after stimulation by GVHR and after LPS-induced polyclonal B cell stimulation are the same. An explanation for the apparent absence of B cells producing IgG antibodies to non-self antigens in F1 mice with GVHD (23, 24) might be that the affinities of antibodies to non-self antigens are so low that the antibodies are only detected in the assay as polyvalent IgM but not as divalent IgG, a difference that may not be detected in oligoclonal serum responses but that becomes apparent when mAbs are measured.

The spectrum of autoantibody specificities is certainly not restricted to intracellular autoantigens that we tested in this paper since we have also seen autoantibodies to cell-surface antigens (Strasser, A., manuscript in preparation). Thus, autoantigen-binding, Ig-secreting hybridomas are formed in all B cell repertoires, whether experimentally unstimulated, or stimulated in vitro or in vivo in various ways. Their frequencies within the IgM-secreting B cell repertoires are indistinguishable from each other. This further supports conclusions drawn by others (1–9) that the B cell repertoire is not purged of self antigen-binding cells. Tolerance within the B cell compartment appears not to be achieved by clonal elimination of IgM-producing cells during B cell development, nor does the system prevent the background activation of self antigen-binding, IgM-producing B cells.

In marked contrast, self antigen-binding, IgG-producing B cells are not detectable, either within the experimentally unstimulated or within the LPS- or SRBC-stimulated repertoires. Therefore, it could be that normally the system does not allow switching of self antigen-reactive B cells to IgG and hence somatic hypermutations in such B cell clones would not occur or would be very infrequent events.

However, if the B cell compartment is stimulated in vivo by allogeneic T cells, self antigen-binding, IgG-producing B cells predominate in the response. This, then, may set the stage for somatic hypermutations and selection of the better fitting self antigen-reactive B cell clones by self antigen. Allogeneic parental Th cells recognizing the foreign class II MHC antigens in the F1 mice probably play a crucial role in the switch from IgM to IgG production. The role
of self antigens in this process of switching to IgG, however, remains unclear from our analyses.

The observation that thymocyte-specific antibodies are readily detectable 1 wk after injection of alloreactive T cells, while antinuclear antibodies can only be detected 2 wks after injection, could indicate that some self antigens might be more efficient in selecting IgG-producing B cell clones than others, perhaps because they are more readily available. The repertoire of self antigen-binding specificities, however, remains largely polyclonal as the GVHD begins to manifest itself. Therefore, no dominance of few self antigens is visible within this autoantigen-binding, IgG-producing repertoire. A possibly significant exception is the detection of one specificity: that against a 160-kD protein in microvilli of kidney brush borders, which we have found only in the GVHD hosts, but not in normal repertoires (Rolink, A., manuscript submitted for publication). In general, our analysis of the autoantigen-binding splenic B cell repertoire of GVHD animals has not yet yielded any definitive clues as to whether a limited selection of autoantigens are instrumental in the induction and/or propagation of this systemic autoimmune disease. It appears worthwhile to expand these repertoire investigations to specific sites within the body where autoimmune stimulation by alloreactive T cells could locally initiate and perpetuate this autoimmune disease. Moreover, experiments aimed at defining the events that are responsible for the switching of self antigen-binding B cells from IgM to IgG production and the role that self antigens play in this process now appear crucial for a better understanding of the pathogenesis of SLE-like and other autoimmune diseases.

Summary

A quantitative analysis of the frequencies of autoantibody-producing B cells in GVHD and in normal mice has been undertaken by generating collections of hybridomas of activated B cells. These hybridomas secreted sufficient quantities of Ig to allow binding analyses on a panel of autoantigens. B cells have been activated in a variety of ways. In vivo they were activated by injection of alloreactive T cells of one parent, leading to GVHD by a foreign antigen, sheep erythrocytes, in a secondary response, or by the polyclonal activator LPS. B cells from an experimentally unstimulated animal were used for an analysis of the normal background. In vitro B cells were activated by alloreactive T cells or by LPS.

The frequencies of hybridomas and, therefore, of activated B cells producing autoantibodies to DNA or to kidney were not significantly different in mice activated by a graft-vs.-host T cell response as compared with B cell populations activated by any of the other procedures. They were found to compose 7.1–17.1% of the total repertoire of activated B cells. Moreover, the frequencies of autoantibody-producing activated B cells does not change with time after induction of the graft-vs.-host reaction. The pattern and frequencies of autoantigen-binding specificities to cytoskeleton, smooth muscle, nuclei, mitochondria, and DNA were not found to be different in any of the groups of hybridomas. The single notable exception, found in GVHD mice, were hybridomas producing autoantibodies to kidney proximal tubular brush border. These results allow the conclusion that autoantigen-binding B cells exist in an activated state in GVHD.
mice, as well as in mice activated by a foreign antigen or by a polyclonal activator, in B cell populations activated in vitro either by alloreactive T cells or by a polyclonal activator, and even in the background of experimentally unstimulated animals. T cell–mediated graft-vs.-host activation, in large part, does not lead to a selective expansion of autoantigen-binding B cells. The main difference between the graft-vs.-host–activated B cell repertoire and all others is that ~90% of the autoantibodies were of the IgG class, whereas all autoantibodies found in the other groups were IgM.

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