Brief Definitive Report

Efficient and Selective Presentation of Antigen-Antibody Complexes by Rheumatoid Factor B Cells

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Summary

Using Epstein-Barr virus B cell clones and antigen-specific T cell clones, we asked how antigen-antibody complexes are handled by B cells. We found that the only B cells capable of efficient presentation of antigen-antibody complexes are those that bind the complexes via membrane immunoglobulin, i.e., rheumatoid factor–producing B cells and, to a lower extent, antigen-specific B cells. On the contrary, nonspecific B cells, although capable of binding antigen-antibody complexes, fail to present them to T cells. Thus, rheumatoid factor B cells can present any antigen in the context of an immune complex and be triggered by T cells specific for a variety of foreign antigens. These results demonstrate a mechanism of intermolecular help that may be responsible for the production of rheumatoid factor and possibly of other types of autoantibodies.

It has been shown that specific antibodies can enhance antigen capture in two ways: (*a*) membrane Igs (mIgs) act as clonally distributed receptors allowing specific B cells to present antigen at very low concentrations (1–4); and (*b*) secreted IgG bound to antigen in immune complexes can enhance antigen presentation by macrophages through Fc-γ receptors (FcγR) (5, 6).

We were interested to understand how the formation of immune complexes, a physiological event in the immune response, might influence antigen capture and presentation by B cells. B cells can bind antigen-antibody complexes in two ways. All B cells, irrespective of their antibody specificity, can bind IgG-immune complexes via FcγR. In addition, a minor fraction of B cells can bind antigen-antibody complexes via mIg: these are either antigen-specific B cells that bind to epitopes of the antigen still accessible in the immune complexes, or rheumatoid factor (RF)-specific B cells that bind to epitopes on self IgG. Here, we show that an antigen complexed with antibody is efficiently presented only by those B cells that bind it through mIg, i.e., by antigen-specific and by RF B cells.

Materials and Methods

B and T Cell Clones. PBMC from a donor recently boosted with tetanus toxoid (TT) were transformed with EBV and cloned by limiting dilution as described (3). Anti-TT antibodies or RF were detected by a reverse Western blot technique. Briefly, supernatants of limiting dilution cultures were blotted on nitrocellulose paper and blocked with 0.5% casein. The specific antibodies were detected using either biotinylated TT (a gift of Dr. J. Nagel, RIVM, Bilthoven, The Netherlands) or biotinylated heat-aggregated human IgG followed by avidin-peroxidase (Southern Biotechnologies, Birmingham, AL). After washing, filters were developed using 3,3′-diaminobenzidine tetrahydrochloride. The RF activity of the selected clones was confirmed by testing the supernatants in standard RF ELISAs using plates coated with rabbit IgG. From the same donor, we also isolated two DR6-restricted TT-specific T cell clones as described (3).

Proliferation Assay. 2×10^4 T cells were cultured with 2×10^4 irradiated (6,000 rad) EBV B cells in 200 μl RPMI-10% FCS in 96-well flat-bottomed plates. The cultures were stimulated with different concentrations of TT in the presence or absence of anti-TT antibodies (Schweizerisches Seruminstitut, Bern, Switzerland). In all cases, TT and antibodies were added to the culture wells 30 min before adding the cells to allow complex formation. In some experiments, complexes were formed at a threefold antibody excess and titrated in the cultures. After 2 d, 1 μCi [^{3}H]thymidine (sp act, 5 Ci/mM) was added and the radioactivity incorporated was measured after an additional 16 h by liquid scintillation counting. Data represent the mean of triplicate cultures. The background of B cells alone (<1,000 cpm) was subtracted.

Detection of Cell-bound Immune Complexes. RF-specific or nonspecific B cell clones were pulsed at 0°C for 30 min with immune complexes containing biotinylated TT (1 μg/ml). The cells were washed and stained with PE-avidin (Southern Biotechnologies) and sorted on a FACS 440 (Becton Dickinson & Co., Mountain View, CA).

Results

Selective Presentation of Ag/Ab Complexes by RF-B Cells. To analyze the presentation of antigen-antibody complexes by B cells, we isolated from a healthy donor EBV-transformed B cell clones representing three types of B cells: (*a*) clones with mIgM specific for human IgG (RF B cells); (*b*) clones with mIgG specific for TT; and (*c*) clones with mIgM or mIgG with irrelevant specificities.

We first compared these B cell clones for their capacity to present TT to T cells in the absence of specific antibody. Fig. 1 *A* shows that only TT-specific B cells, which can cap-

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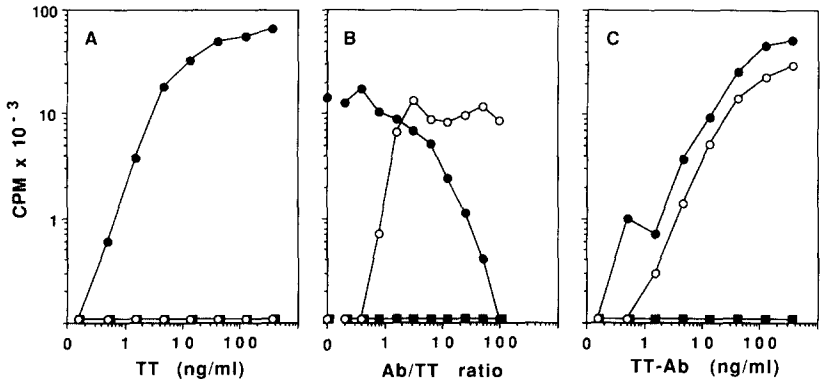

Figure 1. RF-B cells can selectively and efficiently present to T cells TT when complexed with antibody. (*A*) Proliferative response of a TT-specific T cell clone to different concentrations of TT. (*B*) Proliferative response to 10 ng/ml TT in the presence of increasing concentrations of a polyclonal anti-TT antibody. (*C*) Proliferative response to different concentrations of TT complexed with a threefold excess of anti-TT antibody. The values refer to the concentration of TT. The APC were a TT-specific B cell clone (●), a RF-specific B cell clone (○), and a nonspecific B cell clone (■).

ture antigen via mIg, are able to present low concentrations of TT, while RF B cells and nonspecific B cells are not effective at TT concentrations <300 ng/ml.

We then compared the same B cells for their capacity to present a fixed concentration of TT (10 ng/ml) in the presence of increasing concentrations of a human polyclonal anti-TT antiserum. Fig. 1 *B* shows that RF B cells and TT-specific B cells have a strikingly opposite behavior. RF B cells, which were not effective in the absence of antibody, acquired the capacity to present TT in the presence of anti-TT. Presentation by RF B cells was detected at an antibody/TT ratio of 1, was optimal at a ratio of 3, and leveled out at higher antibody concentrations. In contrast, TT-specific B cells progressively lost antigen-presenting capacity upon increase of the concentration of soluble antibody in culture. The high antibody concentration required to inhibit presentation by TT-specific B cells is not surprising, since only a small fraction of the polyclonal antibody will compete with mIg for binding to the same epitope. Fig. 1 *B* also shows that nonspecific B cells did not acquire antigen-presenting capacity in the presence of anti-TT antibody.

Finally, we compared the three B cell clones for their capacity to present different concentrations of immune complexes formed at an antibody/TT ratio of 3:1. As evident from Fig. 1 *C*, RF B cells are almost as efficient as TT-specific B cells in presenting antigen-antibody complexes. Similar results were obtained using another independent RF B cell clone and three additional nonspecific B cell clones, as well as polyclonal B cell populations.

Failure of Nonspecific B Cells to Present Surface-bound Antigen-Antibody Complexes. To investigate whether the failure of nonspecific B cells to present antigen-antibody complexes was due to their inability to bind these complexes, we incubated nonspecific and RF B cells at 0°C with immune complexes containing biotinylated TT, stained the cells with avidin-PE, sorted the cells that had bound similar amounts of TT, and tested their ability to trigger TT-specific T cell clones. Fig. 2 shows that, although both cell types had bound similar amounts of TT, only the RF B cells were able to present TT, while nonspecific B cells could not. Appropriate controls showed that nonspecific B cells were able to present TT added in culture at high concentrations (10 μg/ml) (data not shown, and reference 3).

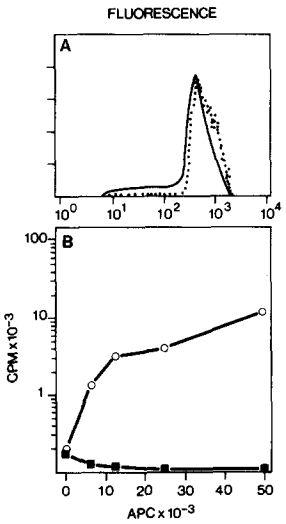

Figure 2. Surface bound TT-IgG complexes are presented only by RF-B cells. RF-specific or nonspecific B cell clones were pulsed with immune complexes containing biotinylated TT (1 μg/ml) stained with PE-avidin, and the cells that bound comparable amounts of TT were sorted. (*A*) FACS analysis of sorted cells: RF-B cells (...); nonspecific B cells (——). (*B*) Stimulation of TT-specific T cells by RF-B cells (○) or nonspecific B cells (■).

Discussion

Our results indicate that the only B cells capable of presenting with high efficiency an antigen (TT) complexed with IgG antibodies are those that bind the immune complexes via mIg, i.e., RF B cells, which bind the Fc portion of IgG, and TT-specific B cells, which bind TT epitopes still available. The failure of nonspecific B cells to present immune complexes most likely bound to FcγR is in agreement with similar reports on mouse B cells (7) and with the fact that the FcγRII isoform on B cells is different from that on monocytes and is not internalized via coated pits (8).

Altogether, these data indicate that in B cells only the mIg-mediated pathway allows efficient uptake of antigen or IgG immune complexes. This provides a quantitative framework for understanding the differential handling of antigen by B cells in the immune response. In contrast to macrophages, B cells are inefficient at capturing antigen by nonspecific means, such as pinocytosis (9) or FcγR (5, 6). Thus, in the absence of antibodies, antigen is selectively captured and presented by macrophages and by specific B cells, leading to T cell priming and to specific antibody secretion. The antibodies

produced form immune complexes that would thus divert antigen from specific B cells and focus it onto macrophages and RF B cells. The latter will thus become the selected B cell target for T cell help. This mechanism explains the negative feedback of antibody on the antibody response (10) and, in addition, the transient production of RF in the secondary immune response or upon immunization with immune complexes (11–13).

Different mechanisms have been proposed to explain the production of autoantibodies, such as polyclonal B cell activation and molecular mimicry. An alternative possibility is that the formation of a complex between self and foreign molecules may result in the focussing of T cell help onto autoreactive B cells. Zinkernagel et al. (14) have recently shown that in vesicular stomatitis virus (VSV)-G protein transgenic mice, the challenge with intact VSV, carrying the "self" G protein physically associated with other viral proteins, results in the production of anti-G autoantibodies. In the case reported here, the association of self IgG with a foreign antigen (TT) has been shown to result in the selective presentation of TT by RF B cells. Thus, the mechanism of intermolecular help (15) (B and T cells recognizing distinct molecules present in the same complex), which is responsible for the production of antiviral antibodies (16), may well be implicated in the generation of an autoantibody response.

It is tempting to speculate that the persistence of immune complexes, caused by inefficient clearance or by local production, may result in sustained T cell help to RF B cells, leading to somatic mutations, affinity maturation, and isotypic switch, which are characteristic of autoimmune diseases (17).

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