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B Lymphocytes Are Crucial Antigen-Presenting Cells in the Pathogenic Autoimmune Response to GAD65 Antigen in Nonobese Diabetic Mice[1]

Marika Falcone, Jae Lee, Gail Patstone, Brian Yeung, and Nora Sarvetnick[2]

Recent reports have shown that B cells play a key role in the pathogenesis of T cell-mediated autoimmune diseases such as insulin-dependent diabetes mellitus (IDDM) in nonobese diabetic mice (NOD). We have investigated the role of B lymphocytes as APCs in the generation of autoreactive T cell responses by comparing spontaneous responses to self Ags in B cell-deficient and wild-type NOD mice. We determined that B cell-deficient mice had no spontaneous responses to 65-kDa glutamate decarboxylase (GAD65), its immunodominant peptides, and the 60-kDa heat shock protein. In contrast, these Ags are able to induce proliferative responses in the splenocyte cultures of B cell-positive NOD mice. However, T cells from B-deficient mice conserved the ability to respond to nonself Ags and mitogens. The Ag-presenting function of B cells was pivotal in the autoimmune response, since the proliferation of wild-type splenocytes to GAD65 was completely inhibited by blocking the surface Ig-mediated capture of the protein Ag by B cells. Responses to immunodominant GAD65 peptides were also absent in B cell-deficient NOD mice, suggesting that B cells are crucial with regard to the diversification of the autoimmune response to various self epitopes. We believe our results represent strong evidence that B cells are required as APCs to generate pathogenic autoimmune T cell responses and provide a direct correlation between the protection from autoimmune diabetes previously reported in B cell-deficient NOD mice and the lack of anti-GAD65 and anti-heat shock protein 60 T cell responses in these mice. ***The Journal of Immunology*****, 1998, 161: 1163–1168.**

Insulin-dependent diabetes mellitus (IDDM)[3] and its experimental model, the nonobese diabetic (NOD) mouse, are clearly T cell-mediated autoimmune diseases (1–3). Although activated inflammatory Th1 lymphocytes are considered responsible for the autoimmune destruction of the target organ (4–6), the cell populations and cytokines associated with humoral immune responses are also major players (7–9). B cell-deficient mice, as well as anti-μ Ab-treated NOD mice, are protected from the onset of IDDM (10–13). Although B cells constitute 30% of the lymphocyte infiltrates in the pancreatic islets of NOD mice (14–16), few studies have analyzed the relevant B/T cell interactions or the function of B lymphocytes as APCs for autoreactive T cells.

It is well known that B lymphocytes are not only involved in humoral immune responses but also have a determinative role as APCs in the generation of T cell-mediated immune responses (17–19). In particular, B lymphocytes are highly efficient APCs for the specific Ag that their surface Igs (sIgs) bind. Resting B cells cannot prime naive T cells, since necessary costimulatory signals such as the B7 molecule are lacking. However, once B cells receive appropriate Ag-specific T cell help through CD40-CD40 ligand, Ag-specific interaction, and cytokine signals (mainly IL-4 and IL-5), they shift to an activated state. Activated B cells express costimulatory signals and can internalize their specific Ag much more efficiently than can non-Ag-specific (professional) APCs such as dendritic cells and macrophages. B cells can receive help from T cell clones that are specific for a single epitope; however, once activated, B cells dramatically increase the uptake and processing of the whole Ag and diversify the T cell response by presenting a broader array of different peptides within the same protein (20).

The latter ability of B cells led to speculation about their importance in amplifying and diversifying T cell-mediated autoimmune responses, allowing the recruitment of diverse populations of self-reactive T cells. Presumably, these events follow the activation of B cells from a few Th clones primed by professional APCs (21). We investigated this hypothesis by analyzing the role of B lymphocytes as APCs in generating spontaneous autoreactive T cell responses to the major autoantigens identified in NOD mice. We tested the autoantigens glutamate decarboxylase (GAD65) and the 60-kDa heat shock protein (HSP60), because spontaneous responses to these Ags were integrally linked with IDDM in both humans and NOD mice. Significantly, the finding that tolerance to these autoantigens ameliorates autoimmune diabetes in NOD mice is the most significant evidence of the involvement of such autoantigens in the pathogenesis of the disease (22–25). These responses in wild-type (wt) NOD mice with an intact population of B cells were compared with those in genetically B cell-deficient NOD mice, and it was determined that the lack of B cells dramatically affected the profile of spontaneous autoimmune T cell responses against GAD65 and HSP60. T cells from the two groups of mice were immunologically responsive and reacted similarly to

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[3] Abbreviations used in this paper: IDDM, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; GAD65, 65-kDa glutamate decarboxylase; HSP60, 60-kDa heat shock protein; PPD, purified protein derivative; wt, wild-type; sIg, surface Ig; MACS, magnetic activated cell sorter.

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a foreign Ag, purified protein derivative (PPD), and to the T cell mitogen Con A. We also found that splenocytes from B cell-deficient mice showed no proliferative response to the peptide determinants of GAD65 that are recognized by T cells from wt NOD mice (23). We believe that these results suggest that B cells have a critical role in vivo in the uptake and processing of the GAD65 protein Ag and in shaping the T cell repertoire to this critical autoantigen. The absence of B cell-mediated diversification of the autoimmune T cell response and the consequent lack of activated, autoreactive T cell clones specific for GAD65 and HSP60 may be the ultimate cause of the protection from autoimmune diabetes that was previously reported in B cell-deficient NOD mice.

Materials and Methods

Mice

NOD mice carrying in their genome a targeted disruption of the membrane exon of the Ig μ-chain gene (μMT) (26) were kindly provided by Dr. D. Mathis (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France). The mice were bred in specific pathogen-free conditions at the Scripps Research Institute (La Jolla, CA). At the time of our experiments, the μMT mice were backcrossed onto the NOD background for eight generations, so that 90% of the negative and heterozygous individuals developed spontaneous diabetes. Homozygous, B cell-deficient NOD mice were screened by typing their PBLs with two-color flow cytometric analysis (FACScan, Becton Dickinson, San Jose, CA) using FITC-conjugated anti-B220 Ab and phycoerythrin-conjugated anti-CD3 Ab (PharMingen, La Jolla, CA). Negative and heterozygous littermates were used as controls in each experiment.

Ags and Abs

rGAD65 was expressed in a baculovirus expression system, and HSP60 was produced in *Escherichia coli* (24); both were generous gifts of R. Tisch (University of North Carolina, Chapel Hill, NC). Both were affinity-purified using a Ni^{2+}/nitrilotriacetic acid resin (Quiagen, Chatsworth, CA). D. L. Kaufman (University of California, Los Angeles, CA) provided us with peptides 6, 15, 17, 34, 35, and 36 from a set of 38 peptides; each peptide was 20 to 23 aa long and spanned the human GAD65 with 5 aa overlaps (27). PPD, an highly immunogenic nonself Ag, was purified from *Mycobacterium tuberculosis* in the laboratory of B. R. Bloom (Albert Einstein College of Medicine, Bronx, NY). The T cell mitogen Con A was obtained from Sigma (St. Louis, MO).

Purified rabbit anti-murine IgG $F(ab')_2$ Ab (blocking Ab for sIg) was purchased from Accurate Chemical and Scientific (Westbury, NY), and rat anti-murine CD16 (blocking for the FcγIII/IIR) was obtained from PharMingen.

Purification of B cells

B lymphocytes were isolated from the splenocytes of NOD mice using the magnetic activated cell sorter (MACS) magnetic separation system (Miltenyi Biotec, Sunnyvale, CA). Briefly, spleens from 8-wk-old female NOD mice were sieved through mesh, depleted of RBCs by NH_4Cl lysis buffer, and washed with PBS. Splenocytes were bound to magnetic bead-conjugated anti-B220 Abs and sorted through MACS separation columns according to the manufacturer's instructions. Two populations were eluted: B lymphocytes and B cell-depleted splenocytes; their purity (98%) was assessed by FACS analysis using anti-B220 mAb (see above).

Proliferation assays

Spleens from female 8-wk-old B cell-deficient NOD and wt littermates were teased through nylon meshes. The single-cell suspensions of splenocytes were washed and plated at 8 to 10 $\times$ 10^5 cells per well in 96-well microtiter plates in HL-1 serum-free medium (Ventrex, Ventura, CA) supplemented with 100 U/ml penicillin/streptomycin, 2 mM glutamine, and 50 μM 2-β-mercaptoethanol. Protein Ags were added at various concentrations: 2 to 50 μg/ml GAD65, 10 to 20 μg/ml HSP60, 10 μg/ml PPD, and 2.5 μg/ml Con A. Peptides were used at the optimal concentration of 7 μM. The primary cultures were kept at 37°C, 5% CO_2 for 5 days, and 1 μCi of [^{3}H]thymidine per well was added during the last 16 h of culture. Thymidine incorporation was measured by liquid scintillation counting.

Statistical analysis

Pooled data of proliferative indexes from each group of mice were computed as means and compared using the Student *t* test.

Results

Protection from IDDM in B cell-deficient NOD mice correlates with a lack of T cell response to islet Ags

To test whether the reported protection from autoimmune diabetes in B cell-deficient NOD mice correlates with an altered autoimmune response against self Ags, we measured their spontaneous in vitro responses to GAD65 and HSP60 and compared them with

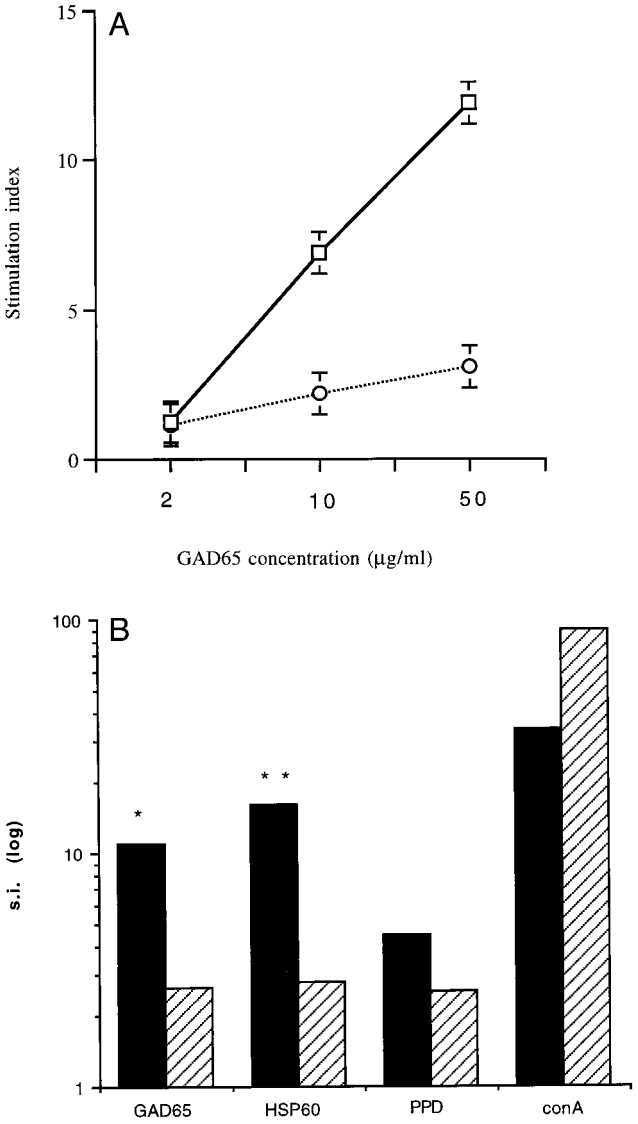

FIGURE 1. B cell-deficient NOD mice lack spontaneous autoimmune responses to self Ags such as GAD65 and HSP60 while maintaining their ability to respond to foreign Ag such as PPD and to T cell mitogen. *A*, A total of 1 $\times$ 10^6 splenocytes from 8-wk-old wt (□) and μMT (○) NOD mice were cultured for 5 days in the presence of graded doses of rGAD65 Ag. [^{3}H]thymidine (1 μCi/well) was added in the last 16 h of culture, and the incorporation of label was measured by liquid scintillation counting. Data from one representative experiment are expressed as stimulation indexes plus the SD of the means calculated from two to three mice that had been tested individually in three separate experiments. *B*, The same number of cells were cultured as described above in the presence of rGAD65 (50 μg/ml), rHSP60 (20 μg/ml), PPD (10 μg/ml), or Con A (2.5 μg/ml). The proliferative responses from the splenocytes of female wt (■) or B cell-deficient (▨) NOD mice that had been tested individually in triplicate cultures were measured as described above, and cumulative data from three separate experiments (2–3 mice/group in each experiment) were expressed as mean stimulation indexes. *$p < 0.0001$; **$p = 0.003$.

those seen in their wt (B cell-positive) littermates. Splenocytes that had been isolated from 6- to 8-wk-old mice were cultured and tested in primary cultures for proliferative responses to the self Ags GAD65 and HSP60. Splenocytes from B cell-deficient mice failed to respond to GAD65 at any of the concentrations tested ($p < 0.0001$) (Fig. 1*A*). The response to HSP60 was also significantly lower in B cell-deficient mice compared with wt mice ($p < 0.004$). However, both groups of mice responded positively and similarly to the highly immunogenic, nonself Ag PPD and to the mitogen Con A (Fig. 1*B*).

B cells are necessary as APCs to activate GAD65-specific autoreactive T cells

To determine whether the reduced spontaneous response to self Ags in B-deficient mice was related to the lack of B cell Ag-presenting function, we isolated B cells from the splenocytes of wt NOD mice and tested the ability of these splenocytes to induce GAD65-specific proliferative responses in vitro. As Figure 2 illustrates, no GAD65 or HSP60 response occurred in the absence of B cells in the B-depleted splenocyte cultures from wt mice. However, adding B cells as APCs to the B cell-depleted splenocyte cultures completely restored the responses to GAD65 ($p < 0.005$) and HSP60 ($p < 0.05$). We also measured the ability of autoantigen-specific B cells from wt mice to stimulate T cells from congenitally B cell-deficient NOD mice. The presence of autoimmune B lymphocytes from wt NOD mice in the splenocyte cultures was sufficient to induce the amplification of GAD65- and HSP60-specific T cell responses in vitro. Interestingly, the immune responses to the foreign Ag PPD (Fig. 2) as well as to the Con A mitogen (data not shown) were not modified by the presence of B cells in splenocyte cultures from both wt and B cell-deficient NOD mice. We believe that these results exclude the possibility that the only function of B cells is to facilitate the uptake of soluble Ags in vitro. In addition, these findings support the hypothesis that activated B cells, which are in vivo committed to recognize their specific autoantigen in the wt NOD mice, are very efficient in vitro in the uptake and presentation of self Ags through their sIg and, consequently, are solely responsible for the amplification of autoimmune T cell responses against GAD65 and HSP60.

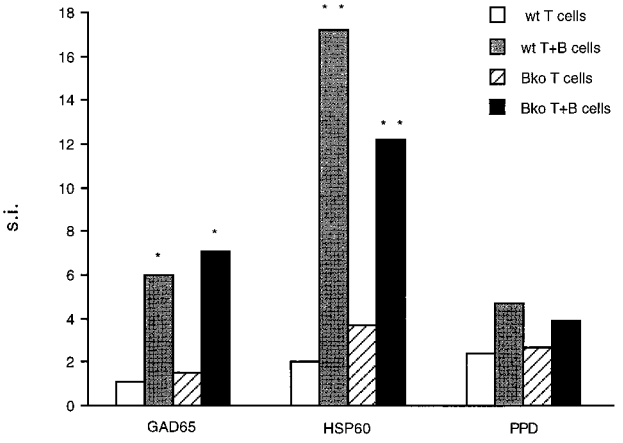

FIGURE 2. B cells are crucial as APCs to induce the GAD65-specific proliferative T cell response. B cells were purified from wt splenocytes by the MACS separation system using anti-B220 Ab and were added in vitro to primary cultures of B-depleted wt or congenitally B cell-deficient NOD splenocytes. The proliferative responses to GAD65, HSP60, and PPD were measured as described in Figure 1. In the absence of B cells, the proliferative response to both autoantigens was almost completely abrogated in the B cell-depleted mice (□), with a stimulation index that was comparable with that of splenocytes from congenitally B-deficient NOD mice (▨). However, the proliferative responses to GAD65 and HSP60 in both groups of mice were restored by the addiction of B cells (2×10^5 per well) to the splenocyte cultures of in vitro B-depleted (▨) and congenitally B cell-deficient (■) NOD mice. The response to the foreign Ag PPD was not significantly altered by the presence of B cells in the splenocyte cultures of both groups of mice. Cumulative data are expressed as mean stimulation indexes from two to three mice that had been individually tested in two separate experiments. B lymphocytes alone did not show any proliferative response when cultured in the absence or presence of Ags (data not shown). *$p < 0.005$; **$p < 0.05$.

T cell response to GAD65 is inhibited by blocking sIg-mediated B cell uptake of the Ag

GAD65-specific Abs that are present in both patients affected by IDDM and NOD mice may participate directly in the pathogenesis of autoimmune diabetes. GAD65-specific Ag-Ab complexes are thought to facilitate the uptake of Ag through FcRs on professional APCs and to help induce autoimmune T cell responses (28–30). Alternatively, GAD65-specific Abs may only represent an epiphenomenon, while specific B lymphocytes directly activate T cell autoimmune responses through their Ag-presenting function by efficiently binding Ag to sIg for subsequent presentation (31). To distinguish between these alternatives and determine how the humoral immune response is involved in the T cell-mediated immune response to GAD65, we interfered with the ability of the IgR of B cells to take up protein Ag by adding anti-F(ab)$_2$ Ab to the primary cultures of splenocytes. As a result, the response of GAD65 was dramatically reduced ($p < 0.06$). In contrast, using an anti-CD16 Ab to inhibit the Fc-mediated capture of the Ag from the FcRs of professional APCs had no effect on the primary response to GAD65 (Fig. 3).

B lymphocytes determine diversification of T cell immune response to peptides of GAD65 in vivo

To exclude the possibility that the B cells were only required in vitro to recall the response to protein autoantigens such as GAD65

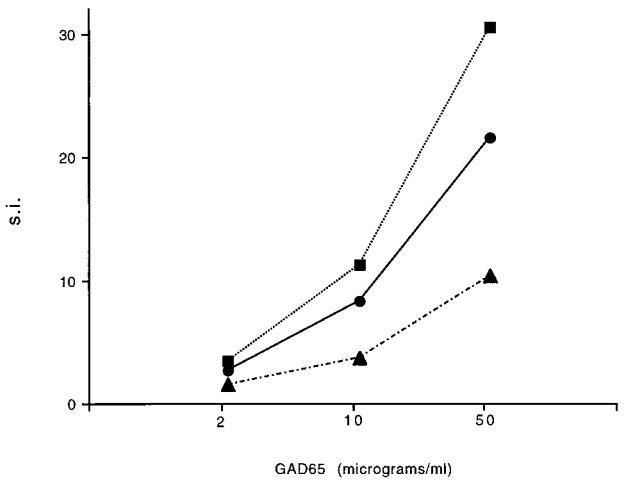

FIGURE 3. The proliferative response to GAD65 is strongly dependent on the sIg-mediated capture of the Ag by B cells. A total of 1×10^6 splenocytes from wt NOD mice were cultured for 5 days in triplicate in 96-well microtiter plates in the presence of different concentrations of rGAD65 (2 to 50 μg/ml). Rabbit anti-murine F(ab)$_2$ Ab to block the sIg of B cells or rat anti-murine FcγIII/IIR (Fc blocking) was added at the beginning of the cultures. After 5 days, proliferation was measured by the mean of [^{3}H]thymidine incorporation. The proliferative responses to GAD65 in the absence of Ab (●) or with anti-CD16 (■) were comparable with those previously observed (see above). Conversely, the response was significantly ($p < 0.06$) inhibited by blocking the Igs on the B cell surface with anti-F(ab)$_2$ (▲). Data are from one of two representative experiments and represent the geometric mean of triplicate determinations.

and HSP60, we looked at the responses to the immunodominant peptides of GAD65. B cells are believed to be particularly efficient in the uptake and processing of their specific protein Ag, while their uptake of peptides and induction of peptide-specific T cell responses is less critical (32). In NOD mice whose splenocytes were in vitro-depleted of B cells, the responses to immunodominant GAD65 peptides 6, 15, and 35 (Fig. 4*A*) were comparable with those of splenocytes from wt NOD mice, confirming that B cells were not necessary to induce these immune responses in vitro. Conversely, genetically B cell-deficient NOD mice did not have measurable responses to these GAD65 peptides ($p \leq 0.05$) (Fig. 4*B*). Evidently, the B cells were important in vivo to shape the T cell repertoire to the self Ag GAD65 and in particular to develop and diversify the autoreactive T cell response to peptide determinants of GAD65.

Discussion

The protection against autoimmune diabetes that has been observed previously in B cell-deficient NOD mice (10–13) clearly correlated here with a dramatic reduction of spontaneous autoimmune responses to GAD65 and HSP60, the two major autoantigens that have been involved in the pathogenesis of the disease. This outcome refutes the long-held hypothesis that B lymphocytes and Th2-type immune responses have no impact on the pathogenesis of autoimmune diseases such as IDDM (5, 6), which are by definition T cell-mediated. In fact, we show here that B lymphocytes are critical to activate autoreactive $CD4^+$ T cells.

The role of B lymphocytes in T cell-mediated autoimmune diseases is still not fully elucidated. Although autoantibodies are detectable in the sera of NOD mice and patients affected by IDDM (33, 34) as well as other T cell-mediated autoimmune diseases (35, 36), their role in the pathogenesis of these diseases remains uncertain. One hypothesis holds that Ag-Ab complexes may facilitate the FcR-mediated capture of autoantigen by professional APCs (28, 30, 37) and increase the processing and presentation of autoantigens. It has also been proposed that autoantibodies may directly bind to pancreatic islets and mediate their destruction by an Ab-dependent cell-mediated cytotoxic response (38). However, there is already convincing evidence that B cells are not required during the effector phase of B cell destruction (39). This finding supports the alternative hypothesis that the secreted autoantibodies only represent an epiphenomenon in the pathogenesis of T cell-mediated autoimmune diseases, while B lymphocytes may play a major role during the induction phase of the disease by directly mediating the activation of autoreactive T cells through their Ag-presenting function (40, 41). Here, we provide evidence that B lymphocytes have a critical role as APCs with regard to the induction of spontaneous autoreactive T cell responses in NOD mice, so that splenocytes from B cell-deficient NOD mice, which have been shown previously to be protected from autoimmune diabetes, did not have any proliferative response to GAD65 and HSP60 in vitro. B cells are known to act very efficiently as APCs by uptaking specific Ag through sIg (18). Activated B cells assumed an Ag presenting function after the specific uptake of their Ag that was 10,000-fold more efficient than that of resting B lymphocytes and professional APCs (21). Our results show that the IgR-mediated uptake of GAD65 Ag by B cells was crucial for the activation of autoreactive T cells, suggesting that Ag-specific B cells have an important role as APCs in the autoimmune response to GAD65 Ag. The possibility that the FcR-mediated uptake of Ag-Ab complexes by professional APCs was responsible for the immune response to GAD65 in vitro was excluded when anti-FcR blocking Ab did not inhibit the response to the self Ag; however, we cannot exclude the possibility that this mechanism may play a role in the uptake of the autoantigen in vivo.

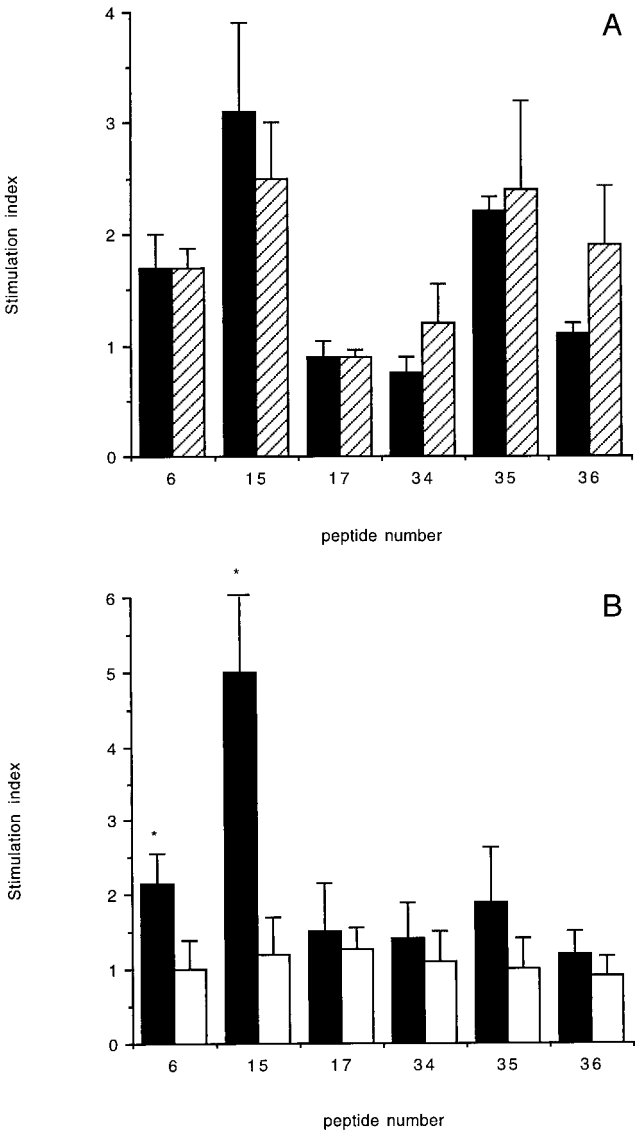

FIGURE 4. B cells are necessary as APCs in vivo to diversify the autoimmune response to GAD65 peptide determinants. A total of 8×10^5 splenocytes from NOD mice (5–9-wk-old) were cultured for 5 days in the presence of 7 μM of peptide. The peptides tested represent a set of six GAD65 peptide determinants that were previously shown to induce spontaneous proliferative responses in NOD mice. Proliferative responses were measured during the last 16 h of culture by the mean of $[^3H]$thymidine incorporation. *A*, In this experiment, the same individual B cell-positive wt mice (■) or in vitro B cell-depleted (▨) mice were tested for their spontaneous immune response to GAD65 peptide determinants. The immune responses to dominant peptides 6, 15 and 35 were comparable in the splenocyte cultures regardless of the presence of B cells, indicating that the Ag-presenting function of B lymphocytes is not critical in vitro for peptide-specific T cell responses. *B*, Splenocytes from B cell-deficient (□) NOD mice showed no proliferative response to the same peptides when compared with wt littermates (■). This finding suggests that B cells were necessary in vivo to generate the spontaneous autoimmune responses against GAD65 peptide determinants. $*p < 0.05$.

Theories about the necessity of B cells to prime T cell-mediated immune responses vary. Some theories propose that B cells are essential as APCs (42–44); others suggest that B cells have no involvement at all (45, 46), or even induce anergy in naive T cells

(47). Resting B cells lack the costimulatory signals necessary to prime T cells, but they become very efficient in the processing and presentation of soluble protein Ags once they receive appropriate help from epitope-specific T cells; they become critical APCs with regard to the amplification of the immune response by priming an increased number of Ag-specific T cell precursors.

Moreover, Ag-specific B cells, owing to their highly efficient uptake of Ag and their processing of Ag-Ig complexes, have the ability to present an array of peptides that are usually not presented (cryptic determinants) by professional APCs (31). Low levels of self Ags may circulate to the thymus and be presented on professional APCs, allowing autoreactive T cells to be deleted. On the other hand, peptide determinants that depend upon prior T cell activation to GAD65 followed by B cell activation, uptake, and processing are not usually available in the thymus, so T cell clones with a high affinity for these peptides may not have been subject to negative selection. As a result, B lymphocytes, by generating these determinants, may allow high affinity autoreactive T cell clones to be activated in the periphery. This hypothesis was strongly supported by the study by Mamula et al., which showed that the Ag-presenting function of B cells is crucial for breaking T cell tolerance to self Ag (20). Particularly, the investigators demonstrated that B cells are responsible for amplifying the T cell response from reactivity with a single immunodominant peptide to reactivity with the entire self Ag (e.g., murine cytochrome *c*). The results presented here provide evidence that B cell-mediated presentation of self Ag represents a key mechanism to break the tolerance against autoantigens such as GAD65 and HSP60 and to generate autoimmune pathogenic T cell responses that ultimately lead to the outcome of autoimmune diabetes. We cannot exclude that the crucial Ag-presenting function of B cells in the autoimmune response to GAD65 and HSP60 may be exclusively due to an amplification of the response through the priming of an increased number of autoreactive T cell precursors, an effect that we were also able to see in vitro by stimulating splenocytes from B cell-deficient NOD mice with in vivo activated, autoimmune B cells from wt littermates. However, the results presented here suggest that B cells as APCs may be also able to amplify the autoimmune response by diversifying the T cell repertoire to the self Ag GAD65. Many studies have shown that B cells are more important for generating immune responses to whole protein Ag rather than to peptide Ag (32). Accordingly, we found that splenocytes taken from wt NOD mice and depleted of B cells in vitro showed a normal profile of immune responses to GAD65 peptides. Conversely, T cells from genetically B cell-deficient NOD mice did not respond to GAD65 peptides, suggesting that, in the absence of B cells, there is a lack of activation of self-reactive T cell clones that are specific for the immunodominant peptides of the GAD65 autoantigen.

To further demonstrate the crucial role of B cells as APCs in the generation of pathogenic autoimmune responses in the NOD mice, we also grafted B lymphocytes that had been purified from wt NOD mice to 3-wk-old B cell-deficient animals. We found that most of the B cell-grafted mice rejected the B lymphocytes; in few cases, we found circulating B cells after 18 h, and none of the grafted animals showed any circulating B cells at 4 wk of age (after 7 days). However, we found that the B cell-grafted mice had an increased insulitis index and most importantly an increased GAD65-specific autoimmune response compared with the B cell-deficient littermates (data not shown). In these mice, the spontaneous response to the GAD65 soluble Ag was still compromised in vitro due to the lack of B cells in their splenocytes at the time of the experiment (7 wk of age). However, the immune responses to GAD65 in the B cell-grafted mice were more similar to those of wt NOD mice rather than B cell-deficient mice. Taken together, these results led us to conclude that B cells were crucial in vivo to generate autoimmune responses to an array of GAD65 antigenic determinants. We can imagine an hypothetical scenario in which a first wave of T cells is primed by professional APCs presenting a self peptide (more likely a molecular mimic peptide) that binds to low avidity T cell clones. These primed autoimmune T cells would subsequently act as helpers for epitope-specific B cells, which in turn would play the crucial role of taking up the whole self protein. Ultimately, the activated self-specific B cells would amplify the autoimmune response by presenting a new set of epitopes to high affinity autoreactive T cell clones that consequently would be empowered to initiate autoimmune diabetes.

We believe that the Ag-presenting function of B cells is crucial only with respect to the generation of autoimmune T cell responses. That is, B cells process self protein Ag and prime high affinity autoreactive T cell clones with self determinants that are not usually expressed on professional APCs in the periphery as well as in the thymic compartment. We found that T cell clones specific for the foreign Ag PPD could be primed entirely without B cells. In that case, B cells were not needed, since APCs such as macrophages and dendritic cells were able to express these foreign determinants to high affinity T cell clones that, since they were nonself reactive, were not deleted in the thymus.

Recently, the Th1/Th2 paradigm applied to the pathogenesis of T cell-mediated autoimmune diseases has been strongly questioned. Several studies have shown that the cell populations and cytokines of the Th2 humoral immune response are deeply involved in autoimmune diseases (7–9). Our study has identified the mechanism by which the humoral immune response, in particular autoantigen-specific B cells, play a critical role in the pathogenesis of IDDM. The lack of amplification of the autoimmune response against a self Ag such as GAD65 by B lymphocytes via their Ig-specific Ag-presenting function can entirely account for the protection from autoimmune diabetes observed in B cell-deficient NOD mice.

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