Brief Definitive Report

INDUCTION OF IMMUNOLOGICAL TOLERANCE REQUIRES THAT THE B CELLS CAN RESPOND TO THE POLYCLONAL B-CELL-ACTIVATING PROPERTIES OF THE THYMUS-INDEPENDENT ANTIGENS*

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Immunological tolerance to thymus-independent (TI) antigens can be readily induced by the injection of supraimmunogenic doses of the antigens. Although tolerance to certain TI antigens, such as lipopolysaccharide (LPS) can be reversed by transferring tolerant cells to irradiated animals or by simply incubating the cells in culture in the absence of antigen (1), other TI antigens like dextrans and levans induce a stable nonreversible state of specific tolerance (2). Tolerance to TI antigens like dextran represents a direct effect on the antigen-specific B cells and the participation of suppressor cells has been excluded (3). It has been suggested that B lymphocytes are tolerized when the Ig receptors bind the antigen (signal 1) in the absence of a second signal (4). Here we will show that specific immunological tolerance only affects a particular subset of B lymphocytes, whereas other B cells having the same immunological specificity are neither activated nor tolerized by interacting with the antigen. The variable that determines whether B cells become tolerant to a particular TI antigen is their susceptibility to the polyclonal B-cell-activating property of the antigen.

Results

The TI antigen used was fluorescein isothiocyanate-dextran B512 (FITC-dextran), which was manufactured by Pharmacia Fine Chemicals, Uppsala, Sweden. Three preparations with different mol wt (150,000, 500,000, and 10^7 the latter being native dextran) were tested. They all contained 1 FITC per 200 glucose residues. Mice were rendered tolerant to FITC-dextran by the intraperitoneal injection of 10 mg (with native FITC-dextran) or 100 mg of the antigen (with the other preparations). At 4- to 10 days after tolerance induction the spleens were removed and the number of anti-FITC plaque-forming cells (PFC) was determined by the Jerne and Nordin plaque assay (5). Untreated mice or mice immunized with 100 μg of FITC-dextran served as controls. As can be seen in Fig. 1, (an experiment with native FITC-dextran) tolerance induction resulted in a complete loss of anti-FITC PFC. The spleen cells from these mice were cultivated in vitro with no additions for 24 h in serum-free cultures,

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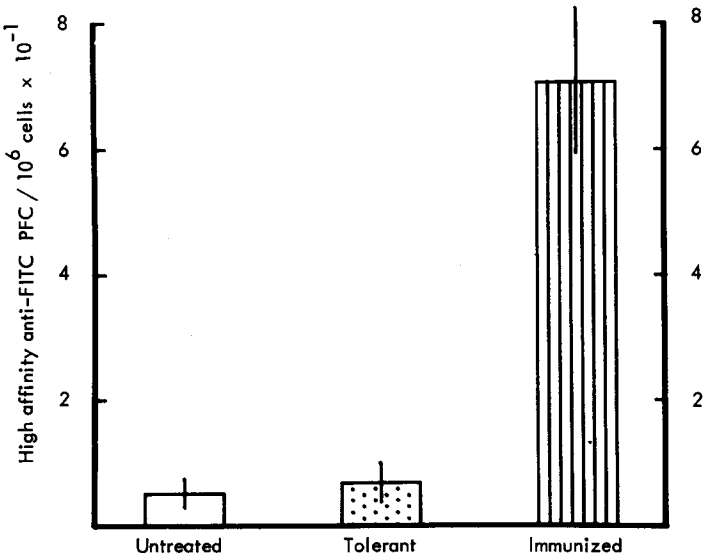

FIG. 1. Anti-FITC PFC in untreated (C3H × DBA)F_1 mice or mice injected 6 days before with an immunogenic (100 μg) or tolerogenic (10 mg) dose of FITC-native dextran. The response (three mice/group) was tested on FITC coated sheep erythrocytes by using a very low concentration of FITC (0.05 mg/ml) to detect cells with high affinity for the antigen, as described before (6).

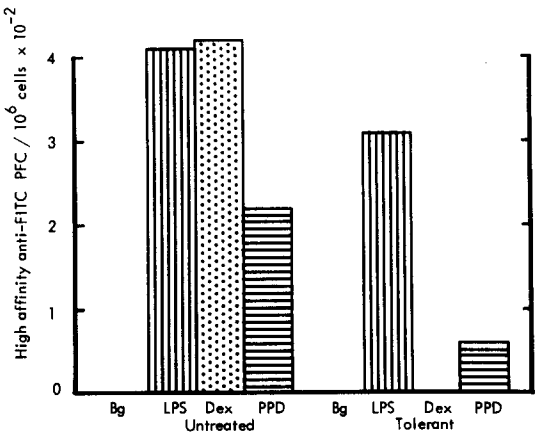

FIG. 2. Cells from the animals in Fig. 1 were cultivated for 24 h in serum-free medium, harvested, and recultivated in 5% human A serum by using 3 × 10^5 cells/ml in 2 ml in 35-mm diameter Petri dishes in the presence of the indicated polyclonal B-cell activators. The following PBA concentrations were used: LPS 100 μg/ml, PPD 100 μg/ml, and native dextran (Dex) 10 mg/ml. The cells were harvested on day 3 and tested against FITC as described in Fig. 1. Bg indicates the response in nonstimulated cultures.

harvested, and recultured in the presence of various polyclonal B-cell activators, such as LPS, purified protein derivative of tuberculin (PPD), and native dextran. The high affinity anti-FITC response was determined after 2 days (by using 10^7 cells/ml) or 3–7 days (by using 3 × 10^5 cells/ml). The results were the same in all culture systems employed and with all MW preparations used as shown in Figs. 2 and 3. It was consistently found that activation by LPS caused

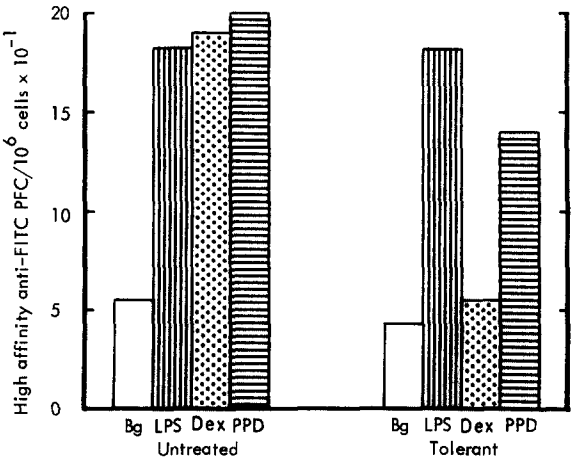

FIG. 3. Cells from 4 R animals tolerized 5 days earlier by an injection of 100 mg FITC-dextran (Dex) 150 were cultivated for 24 h in serum-free medium, harvested, washed, and recultivated in serum-free medium by using 10^7 cells/ml in 35-mm plastic Petri dishes in the presence of 100 μg/ml of LPS, 10 mg/ml of native dextran or 100 μg/ml of PPD. The cells were harvested 2 days later and assayed for high affinity anti-FITC PFC as described in Fig. 1.

the appearance of high affinity PFC against FITC to the same extent with cells from untreated and tolerant mice. PPD was usually less efficient with cells from tolerant animals. In contrast, activation of the cells by polyclonally-activating concentrations of native dextran caused the appearance of anti-FITC PFC only in cultures with cells from untreated and immunized mice, but not with cells from tolerant animals.

Discussion

These findings show that cells from animals rendered completely tolerant to the FITC-dextran conjugate can be activated by LPS and often by PPD to the synthesis of high affinity PFC against the hapten FITC to the same extent as cells from untreated animals. It follows that the anti-FITC reactive cells in the subsets of B cells that can be activated by LPS and PPD had not been made unresponsive to FITC. In contrast, polyclonally-activating concentrations of native dextran failed to activate cells from tolerant animals to the synthesis of anti-FITC PFC. Obviously, only some B cells capable of reacting with FITC were made tolerant of FITC, namely those B cells that could respond to the polyclonal B-cell activating properties of the dextran carrier. Since B cells in different subsets have the same repertoire of V genes (7), the interaction between the antigen and the antigen-binding Ig receptors cannot by itself cause a tolerance signal. Since only the B cells that could respond to the polyclonal-activating property of the antigen were made tolerant, it follows that the polyclonal B-cell-activating (PBA) property of the antigen is responsible for tolerance induction.

These findings have far reaching implications for our understanding of signal delivery in lymphocyte activation and tolerance. They are clearly incompatible with the two signal concept stating that signal 1, which occurs after binding of

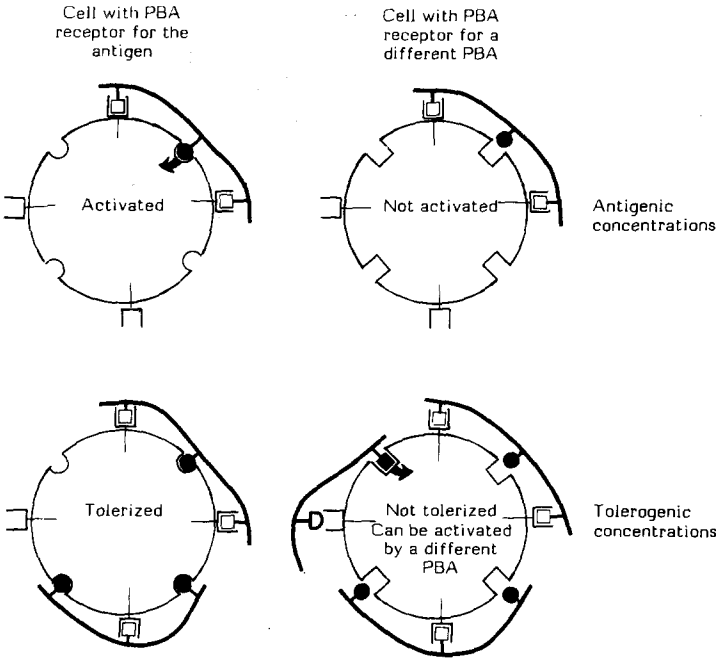

FIG. 4. Schematic summary of the results. FITC-dextran is illustrated as a molecule having two different determinants: the FITC epitope (open squares) and the PBA determinant (black circle). When FITC-dextran binds to the Ig receptor of anti-FITC reactive B cells, only those cells having PBA receptors for dextran will be activated (upper cell to the left), whereas other antigen-specific cells having different PBA receptors are not activated (upper cell to the right). When tolerogenic doses of the antigen is given, the cells with PBA receptors for dextran become tolerized (lower cell to the left), whereas other anti-FITC cells having different PBA receptors are not tolerized (lower cell to the right). These cells can be activated by a polyclonal B-cell activator that can interact with the particular PBA receptor of that subpopulation, as indicated in the figure by a molecule having a different antigenic determinant (open D) and a different PBA determinant (black square).

the antigen to the Ig receptors, results in tolerance induction, whereas signal one plus a second signal which can be delivered by e.g. collaborating T cells or by PBA properties of TI antigen, cause lymphocyte activation. Our experiments show that signal one by itself neither can activate nor tolerize antigen-specific B cells (Fig. 4). Both activation and induction of specific tolerance necessarily required that the B cells possessed receptors for the polyclonal B-cell-activating properties of the TI antigen used as carrier. These findings are only compatible with the one nonspecific signal hypothesis of lymphocyte activation, which states that the activating signal is delivered only by polyclonal B-cell-activating properties of the TI antigen and that the Ig receptors do not signal any event in the B cells, but serve as passive focussing devices that allow the antigen to be focussed selectively to the antigen-specific cells. The selective induction of tolerance in those B cells that respond to the polyclonal B-cell activating properties of the antigen cannot be accommodated by any theory of lymphocyte activation that ascribes a signalling event by the interaction between the antigen and the Ig receptors.

Summary

Mice were rendered specifically tolerant to the fluorescein isothiocyanate-dextran (FITC) epitope by injection of FITC-dextran B512. Their spleen cells were removed at various times and cultivated in vitro with different polyclonal B-cell activators, such as lipopolysaccharide (LPS), purified protein derivative of tuberculin, and native dextran. LPS caused the appearance of high affinity anti-FITC plaque-forming cells to an equal extent with cells from untreated and tolerant animals, whereas native dextran failed to activate cells from tolerant mice, although it was a potent activator of normal cells. It was concluded that tolerance induction only affects those B cells that could respond to the polyclonal B-cell-activating properties of the tolerogen, but not other B cells having an identical set of Ig receptors directed against the tolerogen.

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