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EFFECTS OF INTERLEUKIN 4 ON NEONATAL B LYMPHOCYTE TOLERANCE

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Perturbation of antigen receptors on mouse neonatal B cells by rabbit anti-mouse IgM antibody was shown to inhibit cell proliferation in response to the B cell mitogen lipopolysaccharide. When these antibody-inactivated cells were challenged with lipopolysaccharide in the presence of the helper T cell product interleukin 4, a strong proliferative response was observed. Interleukin 4 alone did not cause proliferation of the antibody-treated B cells. Pretreatment with interleukin 4 did not prevent neonatal B cell inactivation by the antibody. Our results show that neonatal B cells inactivated directly through their antigen receptors can be reactivated by the combined signals of interleukin 4 and lipopolysaccharide.

Direct inactivation of B lymphocyte clones reactive to autologous antigens may provide a basis for self tolerance by the immune system (1). Consistent with this view is the observation that immature B cells with newly acquired sIg are particularly sensitive to inactivation upon engagement of their sIg with either specific antigen or anti-sIg antibody (2). Thus, anti-sIg antibodies used as a polyclonal antigen analog, while inducing a proliferative response in mature adult B cells (3-6), render immature neonatal B cells unresponsive to subsequent challenges with either antigen or the B cell mitogen LPS (7-12). However, the observation that immature neonatal B cells can be induced to respond to antigens in the presence of appropriate helper T cells suggests that direct inactivation of the B cells through sIg can be circumvented by T cell signals (13). In this study, we examined the effects of the helper T cell factor IL 4 on neonatal B cell inactivation by RaMIgM antibody. The results show that, although the antibody treated neonatal B cells fail to proliferate when challenged by LPS, the combined signals of IL 4 and LPS induced significant B cell proliferation.

ABBREVIATIONS

sIg, surface immunoglobulin; RaMIgM, rabbit anti-mouse IgM; LPS, lipopolysaccharide; FBS, fetal bovine serum; IL 4, Interleukin 4.

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MATERIALS AND METHODS

Mice: BALB/c mice were purchased from Charles River Breeding Laboratories (Wilmington, MA). Neonatal (2-3 days of age) and young adult (6-8 weeks of age) BALB/c mice used in this study were bred in our animal facility.

Antibodies and lymphokines: Affinity purified polyclonal rabbit anti-mouse IgM was purchased from Accurate Chemical Company (Westbury, NY.). Monoclonal anti-Thy 1.2 antibody ascites fluid and guinea pig serum used to prepare T cell-depleted spleen cells were purchased from Cedarlane Laboratories (Ontario, Canada). Recombinant mouse IL 4 was purchased from Genzyme (Kneeland, MA) and its specific activity was measured by the supplier using purified murine splenic B cells according to the assay described by Grabstein et al. (14).

Cell culture: Spleens from neonatal and young adult mice were carefully teased apart to give a single cell suspension. Each adult and neonatal spleen generally yielded 1 x 10^8 and 3 x 10^6 cells, respectively. T cell-depleted spleen cells, hereafter referred to as B cells, were prepared by treating spleen cells (1 X 10^8 adult and 2 X 10^7 neonatal cells per ml) with anti-Thy 1.2 antibody and guinea pig complement. This procedure generally caused approximately 50% reduction in viable cell number. B cells (2 X 10^5 /well) were cultured in 96 well round bottom Costar plates (Cambridge, MA) for 72 hrs in 100ul RPMI-1640 medium supplemented with 10% FBS, 2mM L-glutamine, 5x10^{-5} M 2-mercaptoethanol, penicillin, streptomycin and gentamycin. $^{[3]}$H-thymidine was added at a concentration of 1uCi/well 16-18 hrs prior to harvesting of cells. Pretreatment of B cells with either RaMIgM (250 ug/ml) or IL 4 involved a 24-hr incubation with the ligands followed by washing and resuspension of the cells in fresh medium.

RESULTS

Responsiveness of adult and neonatal B cells to RaMIgM and LPS. Fig. 1 shows that adult B cells exhibited a 12-fold increase in thymidine incorporation in response to 100 ug/ml (an optimum dose, data not shown) of RaMIgM. In contrast, no significant proliferative response was induced by RaMIgM

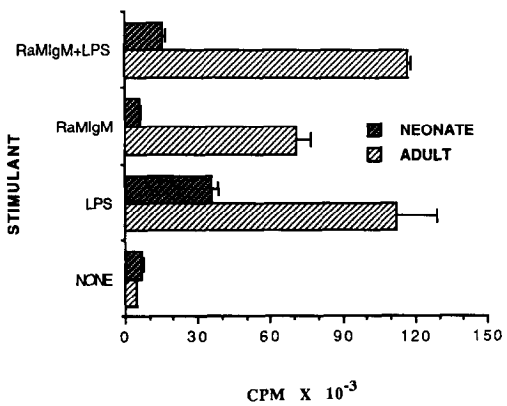

Figure 1: The proliferative response of adult and neonatal B cells to RaMIgM plus LPS. B cells (2x10^6 cells/ml) were cultured with RaMIgM (250 ug/ml) and LPS (40 ug/ml) for 72 hrs. The cells were pulsed with $^{[3]}$H-thymidine (1uCi/well) for the last 16-18 hrs prior to harvesting. The results were obtained in a single representative experiment and are expressed as the mean ± SD of the cpm of quadruplicate wells.

TABLE I

Effect of RaMIgM treatment on the proliferative B cell response to LPS and IL 4

B Cell[a]	Treatment[b]	Stimulant[c]	Proliferation (CPM)[d]
Neonatal	None	None	2,100 ± 760
Neonatal	None	LPS	20,400 ± 320
Neonatal	RaMIgM	None	1,900 ± 760
Neonatal	RaMIgM	LPS	3,800 ± 800
Neonatal	RaMIgM	IL 4	2,200 ± 720
Neonatal	RaMIgM	LPS + IL 4	12,200 ± 1400
Adult	None	None	14,000 ± 5000
Adult	None	LPS	113,500 ± 16400
Adult	RaMIgM	None	13,000 ± 1800
Adult	RaMIgM	LPS	112,000 ± 4400

[a] T cell depleted spleen cells.
[b] Cells were treated with RaMIgM (250 ug/ml) for 24 hrs prior to a 72 hr culture in fresh medium.
[c] LPS and IL 4 were used at 40 ug/ml and 250 U/ml, respectively.
[d] The cells were pulsed with [3]H-thymidine (1uCi/well) for the last 16-18 hrs prior to harvesting. The results were obtained in two representative experiments and are expressed as the mean ± SD of quadruplicate wells.

in neonatal B cells. LPS stimulation of adult and neonatal B cells resulted in 21- and 6-fold increases in thymidine incorporation over the unstimulated cultures, respectively. This difference in the level of LPS-induced proliferation exhibited by the two cell populations correlates well with their respective sIg^+ cell content (data not shown). Therefore, on an individual cell basis, both adult and neonatal B cells appear to be equally responsive to LPS. Costimulation of adult B cells with LPS and RaMIgM did not significantly change the level of thymidine incorporation over that induced by LPS alone. In contrast, when neonatal B cells were challenged with both LPS and RaMIgM, a 50% reduction of the LPS response occurred (Fig. 1).

IL 4 restores LPS responsiveness of anti-sIg tolerized neonatal B cells: Adult B cells treated with RaMIgM for 24 hrs prior to stimulation with LPS were equally responsive to LPS as the untreated cell population (Table I). In contrast, a 24-hr treatment with RaMIgM rendered neonatal B cells completely unresponsive to LPS. When RaMIgM-tolerized neonatal B cells were challenged with both IL 4 and LPS, a 6-fold increase in the proliferative response over the unstimulated control was observed. This response represented approximately 60% of the LPS response exhibited by neonatal B cells that were not exposed to RaMIgM. As noted earlier (Fig. 1), RaMIgM present during LPS stimulation of neonatal B cells inhibited the B cell response by approximately 50%. In the presence of IL 4, this antibody-induced inhibition was reduced to only about 15% (Table I).

The effects of IL 4 pretreatment on RaMIgM inactivation of neonatal B cells are shown in Fig 2. The level of proliferation exhibited by IL 4 pretreated B cells upon challenge with RaMIgM was approximately 2-fold higher than that exhibited by the untreated cell population. However, when IL 4 pretreated neonatal B cells

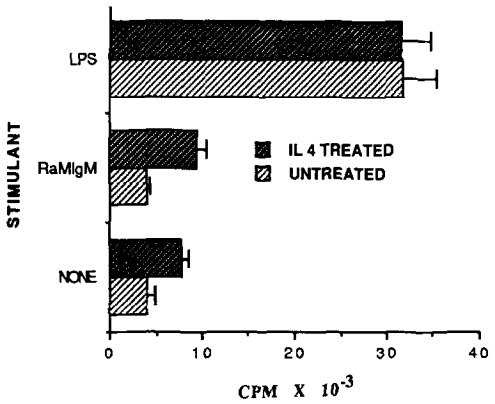

Figure 2: The proliferative response of IL4 treated and untreated neonatal B cells to LPS and RaMIgM. Neonatal B cells ($2x10^6$ cells/ml) were incubated for 24 hrs with or without IL4 (250 U/ml). The cells were subsequently washed and cultured in the presence of LPS (40 ug/ml) and RaMIgM (250 ug/ml) for 72 hrs. The cells were pulsed with $^{[3]}$H-thymidine (1uCi/well) for the last 16-18 hrs prior to harvesting. The results were obtained in a single representative experiment and are expressed as the mean $\pm$ SD of quadruplicate wells.

were challenged with RaMIgM and LPS , no significant increase in the proliferative response due to the antibody was observed. Furthermore, IL 4 pretreatment of neonatal B cells did not change their responsiveness to LPS. These observations demonstrated that a 24 hrs IL 4 treatment of neonatal B cells does not cause maturation of the cells to a state that they can proliferate in response to RaMIgM.

DISCUSSION

Based on observations that lymphokines can restore the immune competence of "tolerized" immature B cells in tumor models (15), the effects of IL 4 on mouse neonatal B cell tolerance were investigated. A 24-hr treatment of neonatal B cells with RaMIgM caused a complete loss of their ability to proliferate in response to LPS. By monitoring the LPS responsiveness, we examined whether RaMIgM-inactivated neonatal B cells could be stimulated with IL 4. This lymphokine has been shown to have a variety of biologic activities in various cell types (16-21). A particularly relevant observation for this study is that IL 4 can have a positive effect on the proliferative response of immune complex inactivated adult B cells (22). Our results showed that RaMIgM inactivated (LPS unresponsive) neonatal B cells can be induced to proliferate by the combined signals of LPS and IL 4. This observation supports the hypothesis that B cells inactivated through crosslinking of sIg are in a state of anergy (23,24) and, when given an appropriate stimulus, they can regain their capacity to respond to a proliferative signal (15,22,23).

It has been established that costimulation of tolerance susceptible neonatal B cells with antigen and antigen-specific helper T cells results in proliferation and differentiation of antigen responsive B cells (7). Our results are consistent with the

possibility that IL 4, a product of helper T cells, may circumvent tolerance induction pathways in high concentrations and permit further proliferation of neonatal B cells. Clearly, more extensive investigation is required to test this hypothesis. Nevertheless, the capacity of IL 4 to interfere with tolerance in immature B cells may be of importance in particular autoimmune conditions typified by autoantibody secretion, B cell hyperactivity, and unusual production of "IL4-like" B cell differentiation and proliferation factors as exemplified by the autoimmune NZB and MRL/lpr mouse strains (24-26).

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