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The Poised B Cell: Lymphokines Induce an Ia-Increase and Antigen-Presenting Function in B Cells

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Individual murine B cells express a wide range of Ia densities on the plasma membrane. Here we demonstrate that a dramatic increase in B-cell Ia could be induced by overnight exposure to an uncharacterized lymphokine (LK). Membrane I-A and I-E molecules were both increased after LK treatment, whereas membrane IgM remained unchanged. Two subpopulations of B cells were identified, based on their requirements for expressing maximal Ia; one subpopulation required only LK, the other required both LK and T cells in the overnight culture. Functional changes accompanied the Ia increase. The functional capacity to present antigens to T cells was lacking in normal resting B cells, but was acquired following LK treatment.

We suggest that the LK-treated B cell has achieved a new differentiation state, one of preparation for interaction with T cells. We term this state the "poised" B cell, and propose that B cells in the poised state may significantly contribute to T-cell activation as antigen-presenting cells. Moreover, poised B cells may themselves find an advantage over normal B cells in successfully acquiring T-cell help.

INTRODUCTION

The major histocompatibility gene complex has been found in many species since its original description as a gene locus controlling immunological rejection of transplanted tissues. Over the past decade considerable effort has been directed toward understanding the role of these gene products in immune responses. The I region of the mouse major histocompatibility complex (reviewed in [1]) encodes the Ia antigens, consisting of two glycoproteins, I-A and I-E, each composed of two peptide subunits. Ia antigens are membrane proteins expressed on some cell types within the immune system, including B cells, macrophages (Mϕ), and dendritic cells, as well as being expressed on some other cell types [2,3]. Ia on cells of the immune system is thought to enable these cells to interact with T cells, which display receptors for Ia.

The density of Ia on cell membranes varies widely among cell types, dendritic cells displaying the most, Mϕ the least, whereas individual B cells express a wide range of Ia densities. The density of Ia, in addition to varying between cells of different lineages, can also be modulated on individual cells. Thus, Mϕ and some other cell types can be stimulated by gamma interferon (IFN-γ) to increase Ia density [4,5], and by endotoxin to reduce Ia [6]. The function of Ia modulation may represent a control over the ability of Mϕ's (and other cells) to interact with T cells. Indeed, the Mϕ-like tumor,

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$P388D_1$, normally expresses no detectable membrane Ia and does not function as an antigen-presenting cell (APC) for T-cell activation. Exposure to uncharacterized lymphokines (probably IFNγ) is reported to induce $P388D_1$ to express membrane Ia, and concomitantly to acquire APC function [7].

In the present study, we report that B cells, like Mϕ, can be stimulated by lymphokines (LK) to express heightened levels of Ia, and that this Ia increase is accompanied by the acquisition of APC function by B cells. Based on these findings, we propose that the resting B cell can be induced by LK to differentiate to a new state, a state of preparation for interacting with T cells—what we term the "poised B cell."

MATERIALS AND METHODS

Mice

C57BL/6J and CBA/J mice were obtained from Jackson Laboratories (Bar Harbor, ME).

Lymphokine Preparation

BALB/c spleen cells (10^7/ml) were cultured for 24 hours in RPMI 1640 and 10 percent fetal calf serum (Dutchland) containing concanavalin A (5 μg/ml, Sigma). Lymphokine-containing supernatants (LK) were then collected, centrifuged, filtered (0.45 micron, Nalge), α methyl-mannoside added (20 mg/ml, Sigma), and frozen at -20°C until use.

B-Cell Purification

B cells were isolated from mouse spleen cell preparations by adherence to goat anti-mouse Ig-coated plates [8]. Nonadherent cells were removed and the adherent fraction was eluted by warming plates for one hour at 37°C, followed by vigorous pipetting. This population was approximately 95 percent B cells as determined by anti-Ig and anti-Ia staining and FACS analysis.

LK Treatment of B Cells

Spleen cells or purified B cells were cultured (10^7/ml) overnight in RPMI 1640 with 10 percent fetal calf serum and 30 percent LK in one ml wells (Costar). In some experiments the purified B cells (5×10^6/ml) were reconstituted with the Ig-coated plate nonadherent cells (5×10^6/ml) prior to LK treatment. Cells were then washed and assayed for I-A, I-E, and IgM expression. To assay their APC function, B cells were similarly cultured (with LK and T cells); following the overnight culture, B cells were purified and assayed for APC function.

Fluorescence-Activated Cell Sorter Analysis

Following the overnight culture with LK, B cells were analyzed for Ia density using the FACS IV system (Becton-Dickinson). Cells were washed in phosphate-buffered saline (PBS) (with 0.02 percent sodium azide) and divided into several staining groups. Cells (3×10^6) were then treated with the following antibodies: (*i*) anti-I-A^k, monoclonal 10-2-16; (*ii*) anti-I-E^k, monoclonal 14.4.4; and (*iii*) anti-IgM, polyclonal rabbit anti-MOPC 104E, purified by affinity to 104E. Cells were incubated with saturating concentrations of antibodies for 30 minutes at 4°C, then washed twice in PBS azide, and resuspended in 100 μl of buffer containing a saturating concentration

of FITC-protein A (Pharmacia) or FITC-rabbit anti-mouse IgG2a (Nordic). Following 30 minutes' incubation at 4°C, cells were washed, resuspended in one ml PBS azide, then kept on ice, and immediately analyzed on the FACS IV. Twenty thousand cells were analyzed for fluorescence intensity.

Antigen Presentation Assay

C57B6/6J mice were immunized with keyhole limpet hemacyanin (KLH) (100 μg, Calbiochem) emulsified in complete Freund's adjuvant (Difco) subcutaneously at the base of the tail. Seven days later, inguinal lymph nodes were removed and cell suspensions prepared. To deplete APC, cells were treated with anti-I-A^b (clone Y3P, [9]) followed by rabbit serum complement (Dutchland) at 37°C for 30 minutes. Cells were then placed in culture (5×10^5/200 μl/well) with KLH (20 μg/ml) in RPMI with 0.5 percent fresh syngeneic mouse serum in microtiter wells (Falcon). APC function of unfractionated spleen cells, B cells, or LK-treated B cells was assessed by treating them with mitomycin C (50 μg/ml, Sigma) for 30 minutes at 37°C, washing, and adding (10^5/well) to APC-depleted cultures of lymph node cells. Proliferation was measured four days later by pulsing with ^{3}H-TdR (1 μCi/well, NEN) followed by harvesting on a MASH apparatus (Microbiological Associates), and counting on a liquid scintillation counter.

RESULTS

LK Stimulates Increased Ia Expression by B Cells

LK regulation of B-cell Ia density was examined using overnight exposure of splenic B cells to LK, followed by quantitation of membrane Ia using FACS analysis. Results are shown in Fig. 1, indicating that untreated B cells displayed heterogeneous densities of Ia. LK treatment resulted in increased densities of IA and IE on B cells. It appears that a subpopulation of B cells (50 to 60 percent) were stimulated by LK to produce maximal levels of Ia, while other B cells remained in the intermediate range of Ia density. IgM density did not increase as a result of LK treatment, indicating a selectivity of the LK effect on expression of membrane proteins.

The LK-induced change in Ia density represents a true increase, rather than a protection from Ia loss during culture, since B-cell Ia density did not diminish during culture in the absence of LK, but retained the Ia density profile of freshly isolated B cells (not shown). It is also unlikely that the overnight culture with LK produced high-Ia B cells by either killing low-Ia cells or stimulating proliferation of high-Ia cells, since cell viability remained good after culture and very little proliferation occurs during this period in cultured resting B cells.

We have identified several T cell lines and tumors which produce potent B cell-Ia-inducing supernatants (not shown). Thus, T cells may be the relevant spleen cell subpopulation producing the LK detected in the studies presented here. As yet, we have no further information on the nature of the relevant LK. However, two reports have recently appeared on the subject of LK induction of B cell Ia. Roehm et al. [10] detected this LK activity in the supernatants of a T-cell hybridoma stimulated with concanavalin A. In their study the molecular weight of the Ia-inducing LK roughly corresponded to that of B-cell growth factor, and tests of interleukin-2 (IL-2) and IFNγ (recombinant DNA products) failed to indicate Ia-inducing activity on B cells. Further support for B-cell growth factor (renamed "B-cell stimulating factor") as the

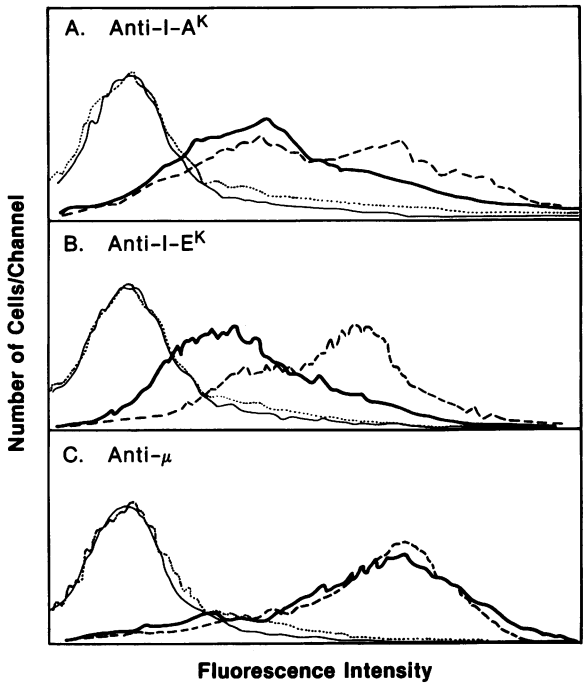

FIG. 1. LK augments Ia expression on B cells. Shown are fluorescence profiles of purified CBA/J splenic B cells following overnight incubation with LK. The LK-treated B cells were stained with the indicated specific reagent (----) or for nonspecific binding of the FITC-coupled second reagent (····). Control B cells (incubated with medium overnight) were also stained specifically (▬▬) or for nonspecific fluorescence (——). Note the LK-induced increase in B cell I-A^k and I-E^k expression with no change in μ expression. Note also that only about one-half of the B cells showed an increase in I-A^k and I-E^k density.

Ia-inducing LK for B cells comes from Noelle et al. [11] who used a relatively pure LK preparation.

T Cells Augment the Ia Induction by LK

As shown in Fig. 1, not all of the purified B cells achieved uniformly high levels of Ia after culture with LK. The same results were obtained when anti-Thy 1 + complement-treated spleen cells were used as a source of purified B cells (data not shown). However, when T cells were included during the LK treatment of B cells (by adding Ig-coated plate nonadherent cells to the purified B cells), Ia density became more uniformly maximized, as shown in Fig. 2. Note that in the absence of T cells, LK treatment produced two populations of B cells which we designated I and II, expressing normal and high Ia densities, respectively. In the presence of both T cells and LK, B cells in zone I were apparently converted into zone II (the T cells can be seen in these plots as the large Ia-negative peak to the left of zone I). In the absence of added LK, T cells induced a small increase in B-cell Ia (not shown).

How might T cells augment the LK action, as observed in Fig. 2? One possibility is that they produce more of the LK, i.e., that we have not used an excess of LK, and that cultured T cells (or T cells activated by the added lymphokines) produce the optimal LK concentration. On the other hand, a T cell may deliver a separate signal, different from the LK, to amplify its effect. In either case, B cells were observed reproducibly to fall into zones I and II, the former requiring both T cells and LK, the latter only LK, to display maximal Ia densities. Although both IA (panel A) and IE (panel B) staining indicated two populations of B cells, we have not yet determined whether the two correspond, i.e., whether B cells with low IA also have low IE and those with high IA also have high IE.

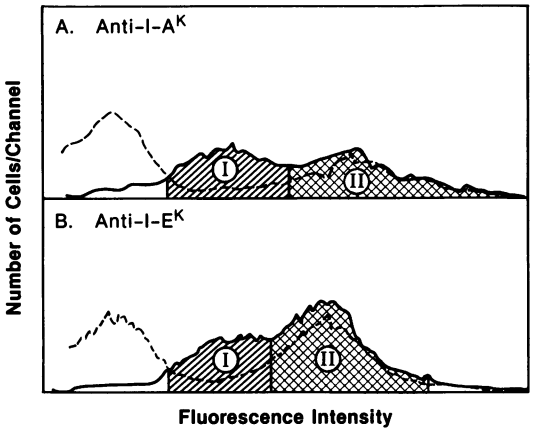

FIG. 2. T cells augment the Ia induction by LK. Purified CBA/J B cells (▬) or B cells + T cells (----) were incubated overnight in medium + LK. Note the distinct populations of B cells with normal (I) and increased (II) I-A^k and I-E^k expression in the absence of T cells. When T cells were included during LK exposure, all B cells showed high I-A^k and I-E^k expression. (B cells in zone I were converted into zone II.)

LK Treatment Induces APC Function in B Cells

What is the functional significance of increased Ia on B cells? Antigen presentation to T cells is known to require recognition of Ia on accessory cells. B cells, although they express Ia, are inefficient APCs. We therefore tested whether LK treatment, which increases Ia, would also improve the APC function of B cells. As shown in Fig. 3, this proved to be the case. Primed LNC were depleted of IA$^+$ cells, resulting in an inhibition of their ability to proliferate when challenged with antigen; various populations of mitomycin-C-treated cells were then tested for the ability to reconstitute proliferation in the APC-depleted LNC. A preparation of unfractionated spleen cells contained APC, as shown, presumably reflecting the dendritic cell or Mϕ content. On the other hand, normal splenic B cells were unable to reconstitute this response, as shown; even when tenfold more B cells were used (not shown), no reconstitution was observed. LK-treated B cells were, however, quite effective in restoring the response, as shown, indicating an acquisition of APC function by B cells.

Since we have not as yet purified either the IA-inducing or APC-inducing LKs, we do not know whether they are the same entity. Nevertheless, that LKs can induce APC function in B cells is an intriguing finding and the observation that Ia increases occur

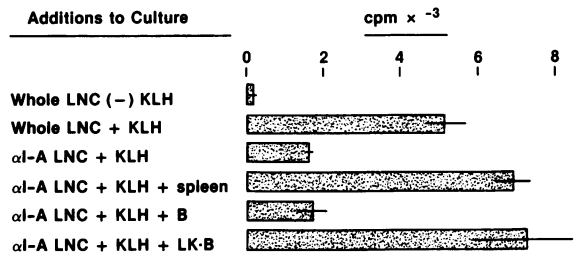

FIG. 3. Acquisition of APC function by C57BL/6 B cells. KLH-primed lymph node cells (LNC) depleted of I-A$^+$ cells (as explained in Materials and Methods) were stimulated to proliferate *in vitro* in the presence of KLH + mitomycin C-treated spleen cells or purified B cells. Note that only whole spleen cells or LK-treated purified B cells presented the KLH to the LNC. Normal, untreated B cells were unable to function as APCs.

concomitantly suggests that the two processes may be related (similar conclusions were recently reached by Roehm et al. [10]).

DISCUSSION

The Poised B Cell

We demonstrate here that B-cell Ia density is not fixed, but can be greatly increased by LK and T cells. What is the functional significance of this Ia increase? Since one function of Ia is thought to be in mediating cellular interactions with T cells [12], it is reasonable to suppose that the high-Ia B cell is poised for such interaction. The ensuing discussion will outline some possible advantages enjoyed by the poised B cell.

One consequence of an Ia increase on B cells may be acquisition of APC function, which, as we show, occurs concomitantly with the Ia increase; but is APC function a physiologically important property of splenic B cells, given the well-established observation that other cells (e.g., dendritic cells [13]) in the spleen are effective APCs and require no prior LK encounter to perform this function? How important is the B cell as an APC? Possibly very important, but not in the spleen. Recent studies indicate that chronic B-cell depletion of mice (by injection of anti-IgM antibodies from birth) results in severe depression of T-cell priming in draining lymph nodes [14]; T-cell priming can be restored in these mice if B cells are injected locally [Ron Y: personal communication]. We therefore speculate that, in lymph nodes, T cells require B cells as APC.

Thus, APC function may be an important, even critical property of B cells, but, as shown in Fig. 3, untreated B cells fail to perform this function. Kakiuchi, Chesnut, and Grey, who first reported APC function by B cells, also noted that resting B cells are ineffective APCs and in their studies required activation by anti-Ig or lipopolysaccharide (LPS) to display APC function [15]. We now show that LK-treated B cells are effective APCs and suggest that it is this later mode—LK activation—by which B cells become APCs *in vivo,* rather than via cross-linking of membrane Ig or by activation with microbial products. Cross-linking membrane Ig would require that the antigen be multivalent—probably an unusual property—and, moreover, would convert only a miniscule proportion of B cells within the population (the antigen-specific clones) to the APC phenotype. The ability of LPS to convert B cells to APC, while a potentially important mechanism during bacterial infection, would not explain the requirement for B cells in the priming of lymph node T cells by protein antigens as discussed above; therefore, we propose that the LK mediates conversion of B cells to APC.

If B cells require the LK to become APC and if the LK is a product of activated T cells, then it is reasonable to assume that, during an immune response, the LK-producing T cells have already had an encounter with an APC prior to secreting the LK. Hence, B cells may function as recruited or "secondary" APC. The advantage of producing additional APC by this mechanism could lie in improving the likelihood of further T-APC encounters or may be required in replacing exhausted "primary" APC; thus, B cells may help expand and sustain T-cell immunity.

We must not assume that the observed increase in B-cell Ia represents the *sine qua non* of APC function. The resting B cell already displays a significant density of Ia—a higher density, in fact, than most Mϕ's in a population capable of APC function. Thus, the observed Ia increase may be coordinated with, but not in itself constitute, the critical characteristic of the antigen-presenting B cell. Another APC function which could arise following LK treatment is the ability to produce IL-1; this product may

perform an essential role in APC function [16,17]. Indeed, it has recently been demonstrated that human B cells can be induced to secrete IL-1-like molecules [18]. Moreover, IL-1 production by Mϕ is controlled by Ia molecules on the cell membrane; these Ia molecules may perform a receptor-like role in receiving T-cell stimuli [17,19]. Hence, by increasing its Ia, a B cell may become more sensitive to T-cell stimuli, resulting in IL-1 production; alternatively, increased Ia production may be coordinated with competence for IL-1 production.

Another APC function which may be lacking in normal B cells could be the ability to process antigens adequately; in the case of protein antigens, this processing may generally involve uptake and proteolytic cleavage, followed by membrane display [20]. Additionally, APC function may involve cell surface molecules (as yet unknown) required for interaction with T-cell membranes. Hence, LK treatment could stimulate, in addition to an Ia increase, any number of properties in B cells, rendering them competent to present antigens; we are currently evaluating several of these potential properties.

Thus far, we have discussed the poised B cell from the point of view of its action on T cells, i.e., APC function. However, the LK-treated B cell, in addition to promoting T-cell immunity as an APC, may also derive selfish benefits. Such poised B cells displaying heightened Ia may have an improved likelihood of contact with helper T cells. Consistent with this notion, it has been previously observed that B cells with high Ia require fewer helper T cells to activate them than do low-Ia B cells [21]. Moreover, we could speculate that a poised B cell with APC function would insure that it is helped by any T cell it encounters (eliminating the need for a third cell type to present antigen) so long as the antigen required for that particular helper T cell is also present. Hence, the poised B cell could activate its own helper T cell, then subsequently, while in the same intimate contact, receive further helper stimuli, driving the B cell to replicate and secrete immunoglobulin.

DEDICATION

As scientist, physician, and scholar, Elisha Atkins is our exemplar. But above all, he inspires us as scientists, by infusing us with an awe and love of nature. For us he has drawn the relationship of the scientist to the natural world as a metaphysical contest pitted between man and his greatest intellectual adversary, the plan of nature. In trying times when petty affairs would break us, it is this romantic view of our profession that will restore us, and that we would hope to transmit in some fractional part to our students after us. It is for this contagious passion that we are most indebted to Elisha Atkins.

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