Interleukin 4 induces membrane Thy-1 expression on normal murine B cells

(immunoglobulin class switching/bacterial lipopolysaccharide/cognate interaction)

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ABSTRACT    Thy-1, a cell-surface glycoprotein of undetermined function, is expressed in relatively large amounts on mouse thymocytes, peripheral T cells, and neurons. It is widely used as a marker to distinguish peripheral T cells from B cells in mice. We show here that, in five distinct mouse strains, recombinant interleukin 4 (IL-4/B-cell stimulatory factor 1) strikingly induces membrane expression of Thy-1 on the vast majority of lipopolysaccharide (LPS)-stimulated normal murine B cells. Thy-1+ B cells are precursors for immunoglobulin-secreting cells. RNA blot analysis indicates that B cells express a Thy-1 mRNA of 1.8 kilobases, the same size as that found in T cells. Cell mixing experiments show that only cells derived from Thy-1−/+ donors express Thy-1, indicating that B cells expressing Thy-1 have not passively absorbed the glycoprotein from another cell source. Recombinant interferon-γ inhibits Thy-1 induction by B cells stimulated with LPS and IL-4. Thy-1 is also induced on B cells that have been stimulated as a result of the specific activation of an IL-4-producing T-helper clone. Anti-IL-4 monoclonal antibody inhibits the induction of B-cell Thy-1 in this T-cell-B-cell interaction.

Thy-1, first described in the mouse (1), is a phosphatidylinositol-linked membrane glycoprotein of undetermined function (2). It bears structural homology to the immunoglobulin variable region domain and is a member of the immunoglobulin supergene family (3). Although Thy-1 has been conserved among a wide array of species, its cellular expression shows considerable variation. In the mouse, Thy-1 is expressed in large amounts on thymocytes, peripheral T cells, dendritic epidermal T cells, and neurons, and in lesser amounts on a number of other cell types including bone marrow-derived pluripotent stem cells and fibroblasts (4). One report demonstrated small amounts of Thy-1 on membrane immunoglobulin-positive cells in the bone marrow (5). In contrast human Thy-1, while expressed on neurons, is absent from the vast majority of thymocytes and is completely lacking on peripheral T cells (6). The rat shows an intermediate cell type distribution in that thymocytes but not peripheral T cells express Thy-1 (7, 8). A proportion of rat B cells also coexpress Thy-1 (9, 10). In the Syrian hamster Thy-1 is readily detected on both B and T cells (11).

Although the function of Thy-1 in vivo is unknown, cross-linkage by anti-Thy-1 antibodies induces murine T-cell activation, which includes an increase in cytotoxic free calcium concentrations and, in the presence of costimulators, results in production of interleukin 2 and proliferation (12–14). Anti-Thy-1 also induces functional changes of neuronal cells in vitro (15).

Interleukin 4 (IL-4) is a T-cell-derived lymphokine with potent effects on cells of virtually all hematopoietic lineages (16). It has striking effects on both resting and activated B cells, including stimulation of proliferation by anti-immunoglobulin-activated B cells (17), up-regulation of expression of class II major histocompatibility complex molecules (18, 19) and of FcεRI (CD23, the low-affinity FcεR) (20–22), and selective stimulation of the production of IgG1 (23) and IgE (24, 25) in vitro, and of IgE in vivo (26).

In the course of experiments on the regulation of immunoglobulin class switching, we observed a striking induction of Thy-1 on lipopolysaccharide (LPS)-activated B cells stimulated with recombinant IL-4. We subsequently observed a similar induction of Thy-1 on B cells stimulated by interaction with an antigen-specific IL-4-producing T-cell clone. The experiments describing these results are reported here.

MATERIALS AND METHODS

Animals, Culture Medium, and Reagents. Pathogen-free 8- to 12-week-old female DBA/2, BALB/c, C3H, C57BL/6, and AKR mice were obtained from DCT Animal Program (National Cancer Institute, Frederick, MD). RPMI 1640 medium (Biofluids, Rockville, MD) was supplemented with 10% fetal calf serum (Flow Laboratories)/2 mM l-glutamine/0.05 mM 2-mercaptoethanol/50 mg of penicillin/ml/50 mg of streptomycin per ml.

Percoll and Ficoll-Paque were obtained from Pharmacia Fine Chemicals. LPS was extracted from Escherichia coli 0111:B4 (Difco) was used at 20 μg/ml in all experiments. Recombinant IL-4 was kindly provided by Immunex (Seattle, WA). Unitage was measured by immunex in an anti-IgM costimulation assay in which 1 unit of activity was equal to ±5 pg of purified IL-4 (27). Rat IgG1 anti-IL-4 monoclonal antibody (mAb) (11B11) (28) was purified from hybridoma supernatant. Rat IgG1 anti-NP mAb (J4-1), a kind gift of R. Coffman (DNAX, Palo Alto, CA), was purified from ascitic fluid. The following purified antibodies were used as staining reagents for flow cytometric analysis: G7 (rat IgG2c anti-Thy-1 mAb; ref. 12), 3H11 (rat IgM anti-Thy-1 mAb; ref. 29, 30H12 (rat IgG2b anti-Thy-1 mAb, obtained from American Type Culture Collection), 2C11 (hamster anti-CD3-e mAb; ref. 30), 6B2 (rat IgG2b anti-B220 mAb; ref. 31), MKD6 (mouse IgG2a anti-Igλ mAb, American Type Culture Collection), Y-3P (mouse anti-λααλααα mAb; ref. 32), MAR 18.5 (mouse IgG2a anti-rat κ, American Type Culture Collection). Phycoerythrin (PE)-labeled goat anti-mouse IgM and PE-labeled normal goat IgG were obtained from Southern Bio-technology Associates (Birmingham, AL). Avidin PE (Phyco-probe) was obtained from Biomedia (Foster City, CA). N-Hydroxysuccinimidoobiotin (Sigma) was conjugated to MKD6 and Y-3P. Fluorescein isothiocyanate (FITC) was obtained from Calbiochem-Behring and was conjugated to

Abbreviations: IL-4, interleukin 4; LPS, bacterial lipopolysaccharide; mAb, monoclonal antibody; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

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RESULTS

IL-4 Induces Expression of Thy-1 on LPS-Stimulated B Cells. In the course of experiments aimed at testing the purity of DBA/2 B-cell populations stimulated with LPS and IL-4, we observed that the majority of the cells were stained by an anti-Thy-1.2 mAb (30H12). To evaluate the significance of this finding, we determined the proportion of cells expressing either B- or T-cell markers in cultures stimulated for 4 days with LPS and IL-4 and quantitated the number of cells expressing membrane Thy-1 using several mAbs specific for different epitopes of the Thy-1 molecule. Resting B cells were cultured at $2 \times 10^5$ cells per ml in the presence of LPS or IL-4 and IL-4 (5000 units/ml). On day 4, cells were stained with mAbs specific for Thy-1 (30H12, G7, and 3H11), CD3 (2C11), B220 (6B2), and Ia (MKD6). The vast majority of cells, arising from 4-day stimulation by LPS and IL-4, expressed Thy-1 (Fig. 1). The intensity of staining of such cells was comparable to the staining of T cells from mesenteric lymph nodes. Comparable numbers of Thy-1-expressing cells were observed after staining with each of the three Thy-1-specific mAbs. None of the Thy-1-specific mAbs gave significant staining of B cells stimulated with LPS only. In separate experiments, IL-4 alone failed to stimulate Thy-1 expression on both small and large B cells (data not shown). No significant expression of the CD3 antigen specific for T cells was observed on cells of either the LPS or LPS and IL-4 group. Staining for the Lyt-1, Lyt-2, and L3T4 antigens was also negative (data not shown). Virtually all cells expressed B220 and Ia antigens after stimulation with either LPS or LPS and IL-4. This latter finding strongly suggests that the vast majority of these cells are B lymphocytes.

Comparable staining with three mAbs specific for different Thy-1 epitopes strongly argues against recognition by these mAbs of a Thy-1 cross-reactive molecule specifically induced by LPS and IL-4. We conclude, therefore, that IL-4 is inducing Thy-1 expression on LPS-stimulated B cells.

B cells stimulated with LPS and IL-4 (5000 units/ml) do not express Thy-1 on days 1 and 2 of culture but show near-maximal expression on day 3 and maximal expression by day 4 (Fig. 2). Thy-1 continues to be expressed at maximal levels for the duration of the 6-day culture. When evaluated on day 4, 150 units/ml is required for detectable Thy-1 expression by LPS-stimulated B cells; maximal expression is observed at 5000 units/ml (Fig. 2).

IL-4 Induces Allele-Specific Coexpression of Thy-1 and Ia on LPS-Stimulated B Cells. To determine whether the Thy-1 expressed on B cells stimulated with LPS and IL-4 is endogenously produced or passively absorbed, we examined the allele-specific expression of Thy-1.2 in mixtures of cells from donors of the Thy-1.2 and Thy-1.1 genotypes. B cells from

![Fig. 1. IL-4 induces expression of Thy-1 on LPS-stimulated normal murine B cells. Resting B cells from DBA/2 mice were stimulated with LPS or LPS and IL-4 (5000 units/ml), harvested on day 4 of culture, and stained with various FITC-labeled mAbs. Viable cells (10^5) were then evaluated by FACS analysis.](image)

![Fig. 2. Time course and dose response for IL-4-induced Thy-1 expression on LPS-stimulated B cells. Resting B cells from DBA/2 mice were stimulated with LPS and various concentrations of IL-4, harvested on day 4, and stained with FITC-labeled anti-Thy-1.2 mAb (30H12). Viable cells (10^5) were evaluated by FACS analysis. In addition, B cells stimulated with LPS and IL-4 (5000 units/ml) were similarly evaluated for Thy-1 expression on days 2–4 of culture.](image)
DBA/2 (Thy-1.2, Ia\(^d\)) and AKR (Thy-1.1, Ia\(^k\)) mice were mixed and stimulated with either LPS or LPS and IL-4 (1000 units/ml). Cells were harvested on day 3 and two-color flow cytometric analysis was performed with combinations of either anti-Thy-1.2 (30H12-FITC) or anti-Thy-1 (G7 plus FITC-MAR) with either anti-Ia\(^d\) (MKD6-biotin plus avidin PE) or anti-Ia\(^k\)(here designated anti-Ia\(^k\))(Y-3P-biotin plus avidin PE). Anti-Thy-1.2 stained Ia\(^d\)+, but not Ia\(^k\)- cells; cells that stained with the anti-Ia\(^k\) failed to stain with anti-Thy-1.2, whereas bright anti-Thy-1.2 staining was observed for negative cells with the anti-Ia\(^k\) mAb (Fig. 3). Bright staining of Ia\(^k\)+ cells with anti-Thy-1, however, indicates that Ia\(^k\) cells are indeed expressing Thy-1. Comparable numbers of Thy-1 Ia\(^k\)-coexpressing cells are detected using either anti-Thy-1.2 or anti-Thy-1. Thus, in cocultures, I-A\(^k\)-bearing cells express Thy-1.2, while I-A\(^k\)-bearing cells, although they are Thy-1+, fail to express Thy-1.2.

This experiment strongly argues that Thy-1 is not passively absorbed by B cells since the Thy-1 allotype expressed by B cells is that anticipated from their genotype, as indicated by their expressed Ia antigens. The allotype-specific coexpression of Thy-1 and Ia strongly argues that the B cell actually synthesizes the Thy-1 it expresses. Furthermore, the finding that the expression of Thy-1.2 is observed in the Thy-1.2 strain DBA/2 but not in the Thy-1.2+ (Thy-1.1+) strain AKR provides additional evidence that we are detecting Thy-1 on B cells and not a cross-reactive protein that shares some epitopes with Thy-1.

RNA Blot Analysis of B-Cell Thy-1-Specific mRNA. RNA blot analysis of total RNA from B cells stimulated with LPS and IL-4 revealed a Thy-1 transcript of 1.8 kb, which is similar in size to the transcript detected in the murine T-cell tumor EL-4 or the murine T-cell line HT-2. No hybridization was detected with RNA from B cells stimulated with LPS alone (Fig. 4).

IL-4 Induces Thy-1 Expression on B Cells in a T-Cell–B-Cell Interaction. We wished to determine whether B cells could express Thy-1 as a result of interaction with an IL-4-producing T-cell clone. To accomplish this, we used cells of the D10.G4.1 line, a cloned T-cell line that produces IL-4 when stimulated by conalbumin and I-A\(^k\)-bearing antigen-presenting cells. To unambiguously evaluate Thy-1 expres-

![Fig. 3. IL-4 induces allele-specific coexpression of Thy-1 and Ia on LPS-stimulated B cells. Resting B cells from DBA/2 (Ia\(^d\), Thy-1.2 genotype) and AKR (Ia\(^k\), Thy-1.1 genotype) mouse strains were mixed in equimolar numbers (1 x 10\(^6\) cells per ml each) and stimulated with LPS and IL-4 (1000 units/ml). Cells were harvested on day 3 and stained simultaneously with both FITC-labeled anti-Thy-1.2 (30H12) or anti-Thy-1 (G7) plus FITC-MAR and biotinylated anti-Ia\(^d\) (MKD6) plus avidin PE or biotinylated anti-Ia\(^k\) (anti-Ia\(^k\), Y-3P) plus avidin PE. Viability cells (5 x 10\(^5\)) were evaluated by two-color FACS analysis.](image-url)

![Fig. 4. IL-4 induces Thy-1-specific mRNA by LPS-stimulated B cells. Total RNA was obtained from B cells stimulated with either LPS alone or with LPS and IL-4 (1000 units/ml), the T-cell tumor EL-4, and the T-cell line HT-2. Total RNA (10 \(\mu\)g) from each group was used for gel electrophoresis. RNA blot hybridization of the resulting nitrocellulose blot was carried out with a \(^32\)P-labeled Thy-1-specific genomic DNA probe. Number on right is in kb.](image-url)

**Fig. 4.** IL-4 induces Thy-1-specific mRNA by LPS-stimulated B cells. Total RNA was obtained from B cells stimulated with either LPS alone or with LPS and IL-4 (1000 units/ml), the T-cell tumor EL-4, and the T-cell line HT-2. Total RNA (10 \(\mu\)g) from each group was used for gel electrophoresis. RNA blot hybridization of the resulting nitrocellulose blot was carried out with a \(^32\)P-labeled Thy-1-specific genomic DNA probe. Number on right is in kb.

**Fig. 5.** IL-4 induces Thy-1 expression on B cells in a T-cell–B-cell interaction. T-depleted spleen cells (1 x 10\(^6\) cells per ml) from C3H (Ia\(^k\), Thy-1.2 genotype) mice were mixed with D10 T cells (3 x 10\(^6\) cells per ml) (Ia\(^k\), Thy-1.1 genotype) with or without conalbumin (100 \(\mu\)g/ml). Anti-IL-4 (50 \(\mu\)g/ml) or anti-NP mAb (50 \(\mu\)g/ml) was added to selected groups. Cells were harvested on day 3 and stained with FITC-labeled anti-Thy-1.2 mAb. Viable cells (10\(^4\)) were evaluated by FACS analysis. AG, antigen.
Table 1. Thy-1+ cells give rise to immunoglobulin-secreting cells

<table>
<thead>
<tr>
<th>Secretion, ng/ml</th>
<th>IgM</th>
<th>IgG1</th>
<th>IgE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS (unsorted)</td>
<td>18,125</td>
<td>175</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>LPS + IL-4 (unsorted)</td>
<td>875</td>
<td>3500</td>
<td>130</td>
</tr>
<tr>
<td>LPS + IL-4 (Thy-1-dull)</td>
<td>350</td>
<td>1400</td>
<td>62</td>
</tr>
<tr>
<td>LPS + IL-4 (Thy-1-bright)</td>
<td>400</td>
<td>1600</td>
<td>72</td>
</tr>
</tbody>
</table>

Resting B cells were stimulated with LPS alone or with LPS plus IL-4 (1000 units/ml). On day 4, cells from both groups were stained with FITC-labeled anti-Thy-1.2 (30H12). Cells were then sorted on the basis of either bright or dull Thy-1 staining. Reanalyses of sorted cells demonstrated that >96% of the cells correctly expressed the cell-surface phenotype for which the sorter had been programmed. Sorted and unsorted cells, previously stimulated with either LPS or LPS plus IL-4, were then reintroduced into culture in the continued presence of LPS or LPS plus IL-4, respectively. Two days later, culture supernatants were removed for measurement of immunoglobulin isotype concentrations by ELISA.

LPS and IL-4. Supernatants obtained from both groups, at the end of culture, contained IgM, IgG1, and IgE in comparable amounts (Table 1). These findings therefore establish that Thy-1 is not only expressed on cells with B lymphocyte phenotypic markers but also on precursors of immunoglobulin-secreting cells.

Interferon-γ Inhibits IL-4-Induced Thy-1 Expression by LPS-Stimulated B Cells. Interferon-γ inhibits virtually all effects of IL-4 on murine B cells. Interferon-γ strikingly inhibited the IL-4-induced Thy-1 expression by LPS-stimulated B cells (Fig. 6). Neither interferon-γ nor IL-2 alone stimulated Thy-1 expression by LPS-stimulated B cells (data not shown).

DISCUSSION

Although the existence of Thy-1 on a variety of cell types has been known for almost a quarter of a century, its function is unknown. Cross-linking of Thy-1 by anti-Thy-1 mAb causes increases in cytosolic [Ca2+] in T cells and Thy-1-transfected B cells and costimulates lymphokine release and proliferation (12–14, 39). Anti-Thy-1 has also been shown to induce regeneration of processes in cultured rat ganglion cells (15). Recently, transgenic mice possessing a Thy-1 gene ligated to the immunoglobulin heavy-chain enhancer were shown to express Thy-1 on their B cells and developed B-cell lymphoproliferation in bone marrow and peripheral lymph node (40). It has also been noted that anti-Thy-1 mAb partially inhibited T-cell-B-cell conjugate formation as a result of antigen-dependent class II major histocompatibility complex restricted linkage of these two cell types under conditions in which other antibodies to various cell-surface determinants were ineffective (41). Collectively, the findings described above suggest the possibility of an important function for Thy-1 in signal transduction and/or cellular communication in vivo. The cell-type-specific expression of Thy-1 and its regulation during the ontogeny of certain cell lineages further highlights its possible physiologic significance. The possible in vivo presence of Thy-1-expressing B cells in mice, after specific immunization, may be reevaluated in light of our present findings.

We have previously demonstrated that IL-4 is critical for IgE production in mice injected with anti-IgD or infected with Nippostrongylus brasiliensis (26). Furthermore, it has been shown that the FceRI, the low-affinity Fce receptor, is strikingly induced on B cells by culture with LPS and IL-4 (42). The coordinate expression of these molecules under conditions that favor IgG1 and IgE secretion in vitro and IgE secretion in vivo raises the possibility that Thy-1 on B cells may serve to regulate the differentiated functions of these cells.

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