INTERLEUKIN 5 AND INTERLEUKIN 2 COOPERATE WITH INTERLEUKIN 4 TO INDUCE IgG1 SECRETION FROM ANTI-Ig-TREATED B CELLS

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Exposure of murine splenic B cells to insoluble anti-Ig for 48 h yields low density B cell blasts (anti-Ig blasts) that proliferate and secrete IgM when recultured with either LPS or T cell–derived lymphokines (1, 2). Culture of anti-Ig blasts with a mixture of T cell–derived lymphokines that includes IL-4 (EL4 Sn) (1, 2) results in secretion of IgG1, which is blocked by monoclonal anti-IL-4 (1). Thus, IL-4 can alter Ig isotype expression in B cells pre-activated by anti-Ig. However, IL-4 alone does not sustain proliferation nor promote differentiation of anti-Ig blasts (1, 2), suggesting that other lymphokines are required to enable IL-4-induced IgG1 secretion. We have examined the effects of other T cell–derived lymphokines on IL-4-mediated IgG1 secretion. While IL-4 was capable of suppressing IgM secretion from LPS-stimulated anti-Ig blasts, secretion of IgG1 required lymphokines in addition to IL-4, including IL-5 and IL-2.

Materials and Methods

Mice. BALB/c mice were obtained from Cumberland Farms, Clinton, TN and used at 6–12 wk of age.

Reagents. Affinity-purified antibodies were obtained from Jackson Immunoresearch, Avondale, PA (goat anti-mouse IgM + IgG, and anti-μ chain and anti-γ chain), and from Southern Biotechnology Associates, Birmingham, AL (goat anti-IgG1, and goat anti-κ). Myeloma standards were obtained from Litton Bionetics, Charleston, SC (MOPC 21). Monoclonal rat anti-IL-4 (11B11) was generously provided by Dr. E. S. Vitetta (Southwestern Medical School, Dallas, TX). Radiochemicals were obtained from New England Nuclear, Boston, MA. *Salmonella typhosa* LPS was purchased from Difco Laboratories Inc., Detroit, MI.

T Cell Supernatants. Lymphokine-containing supernatant (Sn) was obtained from EL4 cells as previously described, except serum-free conditions were used (3). IL-4-depleted Sn, [(D)EL4 Sn] was prepared by twice passing EL4 Sn over an anti-IL-4 affinity column (11B11-Sepharose, see below).

Lymphokines. rIL-2 was provided by Dr. J. Farrar (Hoffman-LaRoche, Inc., Nutley, NJ). rIL-5 was produced in Xenopus oocytes, as described (4), using a plasmid containing IL-5 cDNA provided by Dr. T. Honjo (Kyoto University, Kyoto, Japan). 1 U of IL-5 was defined as the amount of IL-5 required for 1/3 maximal stimulation of [^{3}H]thymidine incorporation by BCL_1 cells. IL-4 was affinity purified from EL-4 Sn using a 11B11-Sepharose column as described by Ohara et al. (5). 1 U of IL-4 was defined as the amount of IL-4 required

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for 1/3 maximal stimulation of [^{3}H]thymidine incorporation by splenic B cells cultured with anti-Ig-sepharose. 1–5 U/ml IL-4 stimulates maximal IgG1 secretion from LPS-stimulated B cells.

B Cell Preparations. B cells were prepared by treating spleen cells with anti-Thy-1 and L3T4 followed by lysis with baby rabbit serum (Pel-Freeze Biologicals, Rogers, AR). In some experiments splenic B cells were isolated by panning on anti-Ig-coated plates. High density B cells (1.081–1.086 g/ml) were isolated on discontinuous Percoll density gradients as described (2).

Cell Culture. Anti-Ig blasts were prepared from high density or unfractioned splenic B cells isolated by centrifugation (400 *g* for 10 min) over Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) as described (1, 2). Blasts were washed and recultured (in RPMI 1640, 5% FCS, 5 μg/ml gentamicin, and 50 μM 2-ME) at 2×10^4 cells/0.2 ml with 20 μg/ml LPS in 96-well microtiter plates. All additions were made in triplicate.

RIA. Culture supernatant was assayed for Ig isotypes by solid-phase RIA (1).

Results

Lymphokine Dependence of IL-4-mediated IgG1 Secretion from Anti-Ig Blasts. IL-4 stimulates secretion of IgG1 from resting B cells in the presence of LPS (6–9), and from anti-Ig blasts in concert with other lymphokines (1). We first asked whether LPS and affinity-purified IL-4 were sufficient to elicit IgG1 secretion from anti-Ig blasts. In contrast to results with resting B cells, culture of anti-Ig blasts with LPS and IL-4 at concentrations up to 50 U/ml had virtually no effect on IgG1 secretion (Fig. 1). Addition of T cell-derived lymphokines from IL-4-depleted EL4 Sn [(D)EL4 Sn] stimulated IgG1 secretion from anti-Ig blasts treated with LPS + IL-4. IgG1 secretion in the presence of (D)EL4 Sn and LPS was half maximal at ∼5 U/ml IL-4, and was inhibited by monoclonal anti-IL-4. Secretion of IgG1 from anti-Ig blasts cultured with IL-4 + (D)EL4 peaked at 6 d of culture, whereas LPS + IL-4 failed to enhance IgG1 secretion when secondary cultures were extended to 8 d (not shown). In the absence of IL-4, (D)EL4 Sn enhanced background IgG1 secretion an average of twofold in five experiments. Cell recoveries at the end of 5-d cultures were also increased 50–100% by (D)EL4 Sn (data not shown).

rIL-5 and rIL-2 Cooperate with IL-4 to Stimulate IgG1 Secretion from Anti-Ig Blasts. We

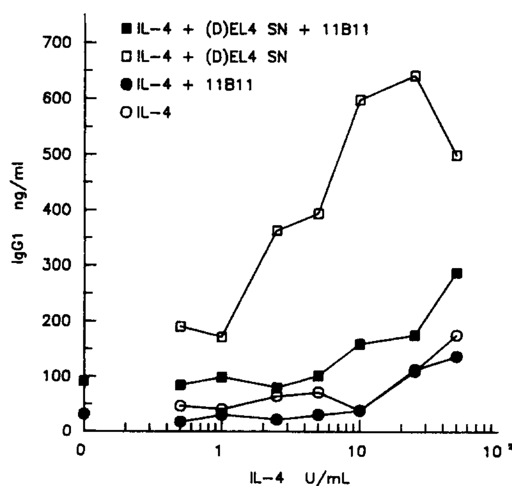

FIGURE 1. IL-4-mediated enhancement of IgG1 secretion is dependent on other T cell-derived lymphokines. Anti-Ig blasts were prepared from high density B cells as described in Materials and Methods, then recultured at 10^5/ml with LPS (20 μg/ml) or LPS + (D)EL4 Sn (5 μl) for 6 d. 11B11 (monoclonal anti-IL-4) = 1 μg/ml. Results presented are the mean of triplicate wells from a representative experiment.

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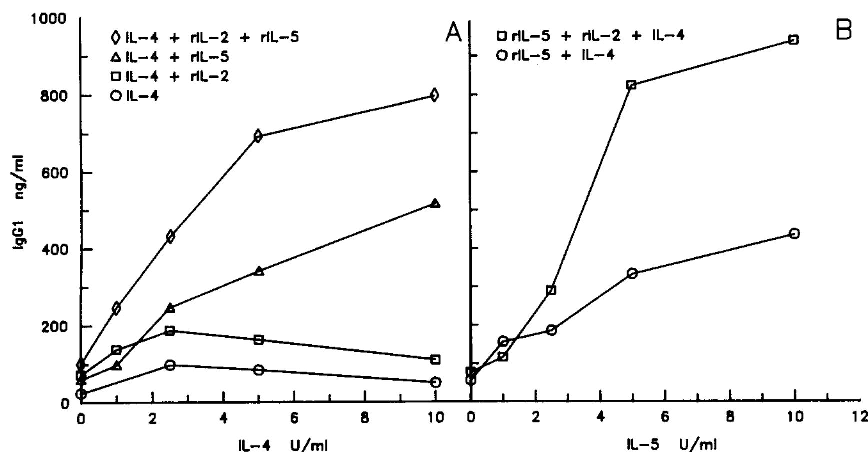

FIGURE 2. rIL-5 and rIL-2 stimulate IL-4-dependent IgG1 secretion from anti-Ig blasts. Anti-Ig blasts prepared from high density B cells (*A*) or from cells that adhered to anti-Ig-coated plates (*B*) were recultured with LPS (20 μg/ml) + lymphokines for 5 (*A*) or 7 d (*B*). (*A*) rIL-2 = 20 U/ml; rIL-5 = 10 U/ml. (*B*) IL-4 = 5 U/ml; IL-2 = 50 U/ml.

next wished to identify the lymphokine(s) in EL4 Sn that allowed IgG1 secretion from anti-Ig blasts. Two lymphokines present in EL-4 Sn that have demonstrated activity on B cells are IL-5 and IL-2. Since IL-2, IL-4, and IL-5, alone or in combination (Simpson, L. G., J. M. Purkerson, and P. C. Isakson, manuscript in preparation), stimulate scant proliferation or Ig secretion from anti-Ig blasts, it was necessary to include LPS as a mitogen in these experiments. Thus, anti-Ig blasts were cultured with LPS and IL-4, in combinations with rIL-5 and rIL-2. Neither rIL-2 or rIL-5 enhanced background IgG1 secretion in the presence of LPS (<100 ng/ml). In contrast, when combined with 5 U/ml IL-4, rIL-5 stimulated IgG1 secretion up to 10-fold (Fig. 2, *A* and *B*); half maximal responses occurred at ~3 U/ml of rIL-5. Stimulation of IgG1 secretion by rIL-2 plus IL-4 was variable, ranging from no effect to a 2–3-fold increase in IgG1. However, addition of rIL-2 consistently increased the IgG1 response to rIL-5 + IL-4 by 2–3-fold. The IgG1 response to the combination of rIL-2, IL-4, and rIL-5 ranged from 40 to 100% of that obtained with crude EL-4 Sn (not shown). Cell recoveries from 5-d secondary cultures were increased <50% by rIL-5 + rIL-2.

IL-4 Suppresses IgM Secretion from Anti-Ig Blasts. The preceding experiments suggest that anti-Ig blasts may be unresponsive to IL-4 in the absence of other lymphokines. Since IL-4 has been shown to markedly suppress IgM secretion from LPS-stimulated B cells (8), we asked whether IL-4 could exert this effect on anti-Ig blasts. IgM secretion from LPS-treated anti-Ig blasts was strikingly inhibited by IL-4 (Fig. 3, *A* and *B*) over the same concentration range that stimulated IgG1 secretion in the presence of other lymphokines (compare with Figs. 1 and 2); IgM suppression was reversed by anti-IL-4. Cells cultured with (D)EL-4 Sn and LPS produced two-fold more IgM than cells cultured with LPS alone; in the presence of both LPS and (D)EL4 Sn, IL-4 suppressed IgM secretion by >80% (Fig. 3 *B*).

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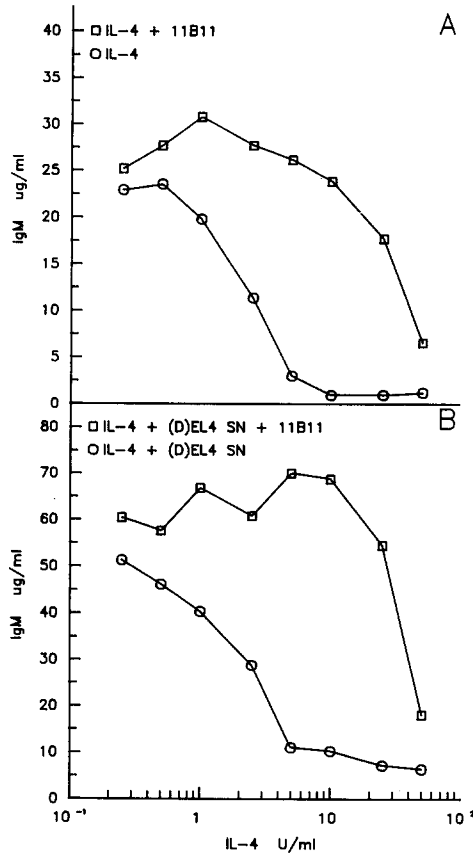

FIGURE 3. IL-4 suppresses IgM secretion from anti-Ig blasts. Anti-Ig blasts prepared from high density B cells were recultured with LPS (20 μg/ml) (*A*) or LPS + (D)EL4 Sn (*B*). 11B11 = 1 μg/ml.

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Discussion

The present findings indicate that B cells pre-activated by anti-Ig differ from resting B cells with respect to lymphokine requirements for isotype switching to IgG1. Although IL-4 (in the presence of LPS) stimulates IgG1 secretion from resting B cells (6–9), additional T cell–derived lymphokines were required to obtain IgG1 secretion from anti-Ig blasts. In addition suppression of IgM secretion by IL-4 was clearly dissociated from its enhancement of IgG1 secretion on anti-Ig blasts.

Several explanations appear plausible for the differences in lymphokine requirements for IgG1 secretion observed between B cells and anti-Ig blasts. Pretreatment with anti-Ig may prevent some of the actions of IL-4, so that the requirement for IL-5 may be unique to anti-Ig pretreated B cells. Alternatively, LPS and anti-Ig may stimulate distinct B cell subsets that have different lymphokine requirements for Ig isotype regulation. The subset of B cells that secretes IgG1 in response to LPS + IL-4 may have received prior stimulation in vivo (perhaps from T cells), thus allowing isotype regulation by IL-4 in the absence of other lymphokines. We have noted that anti-Ig blasts cultured with LPS secrete lower quantities of all IgG isotypes (but not IgM) than do LPS-treated splenic B cells, consistent with the hypothesis that distinct B cell subsets are stimulated by LPS vs. anti-Ig.

The mechanism(s) by which IL-5 (and IL-2) facilitates IL-4-mediated IgG1 secretion is not clear. The observation that IL-4 alone suppresses IgM secretion (Fig. 3) demonstrates that anti-Ig blasts are capable of responding to IL-4 in the absence of other lymphokines. Thus, IL-5 may promote proliferation and/or Ig secretion from cells precommitted to IgG1 production, as has been shown with IgA secretion from LPS-stimulated Peyer's patch B cells (10). However, IL-5 alone had little effect on either background IgG1 secretion or cell recovery from cultures of anti-Ig blasts, suggesting that IL-5 does more than promote differentiation of precommitted cells. The data presented in Figs. 2 and 3 are consistent with the hypothesis that IL-5 and IL-4 both regulate molecular aspects of isotype switching. Since IL-4 alone is capable of suppressing IgM secretion, it is interesting to speculate that IL-4 may initiate recombinational events that lead to decreased expression of $C_H\mu$, while IL-5 may regulate events that complete IL-4-directed switch recombination to $C_H\gamma1$.

Summary

We have analyzed requirements for IL-4-induced secretion of IgG1 from anti-Ig-activated B cells. Activated B cell blasts prepared by culture of high density B cells with anti-Ig failed to secrete IgG1 upon subsequent culture with LPS and IL-4. However, IL-4 markedly suppressed IgM secretion in the same cultures. Addition of a mixture of T cell–derived lymphokines or rIL-5 to LPS-stimulated anti-Ig blasts restored IL-4-stimulated IgG1 secretion; rIL-2 further enhanced the response to IL-4 + rIL-5. These results suggest that IL-4, IL-5, and IL-2 cooperate in the regulation of B lymphocyte Ig isotype expression.

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