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The Absence of IL-6 Does Not Affect Th2 Cell Development In Vivo, But Does Lead to Impaired Proliferation, IL-2 Receptor Expression, and B Cell Responses

Anne Camille La Flamme and Edward J. Pearce

The role of IL-6 in Th2 cell differentiation and response development after the injection of eggs from *Schistosoma mansoni* was investigated using wild-type (WT) and IL-6−/− mice. IL-6 was induced in the lymph nodes (LN) of WT mice within 24 h of egg injection, and IL-4 production by WT LN cells and CD4 T cells isolated 24 h after egg injection and stimulated in vitro was observed. In the absence of IL-6, this early production of IL-4 by LN cells and purified CD4 T cells was not abolished; although the level of IL-4 produced by IL-6−/− LN cells was similar to WT, IL-4 production by purified IL-6−/− CD4 T cells was reduced compared with WT. Despite the difference in CD4 T cell production of IL-4, the development of egg-specific Th2 cells 7 days after egg injection was not affected by the absence of IL-6. Nevertheless, Ab production was impaired and the in vitro proliferative response of whole LN cell populations, CD4 and CD8 T cells, and B cells from IL-6−/− mice was poor compared with WT. The proliferative defect in the IL-6−/− cells correlated with decreased IL-2R expression, and addition of exogenous IL-6 enhanced IL-2R expression as well as proliferation of LN cells from IL-6−/− mice. These studies demonstrate that Th2 differentiation and response development in vivo is not dependent on IL-6, but that Th-dependent and independent B cell responses are. Our results also emphasize the importance of IL-6 for lymphoproliferation, possibly through induction of IL-2R expression. *The Journal of Immunology, 1999, 162: 5829–5837.*

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3 Abbreviations used in this paper: Thp, precursor Th cell; WT, wild type; LN, lymph node; SEA, soluble egg Ag; CFSE, 5-(and-6)-carboxyfluorescin diacetate, succinimidyl ester; MFI, mean fluorescent intensity; HPRT, hypoxanthine phosphoribosyltransferase.

4 L. Rosa-Brunet, E. A. Sabin, A. W. Cheever, M. A. Kopf, and E. J. Pearce. IL-5 plays a role in the development of IL-4 producing non-T, non-B cells during murine schistosomiasis but is not required for the expression of a Th2 response or host resistance mechanisms. *Submitted for publication.*
during in vivo T cell differentiation, it plays an important function in in vitro T and B cell proliferation and in B cell responsiveness following immunization.

Materials and Methods

Animals, parasites, and experimental inoculations

Six- to eight-week-old, male or female, wild-type (WT) hybrid (C57BL/6 × SV129) (#010405) and (C57BL/6 × SV129) IL-6−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). S. mansoni (Puerto Rican strain NMRI) eggs were isolated from the livers of infected mice, washed extensively, resuspended at 50,000 eggs/ml in low endotoxin PBS (Sigma, St. Louis, MO), and stored at −70°C until used as previously described (19, 20). Mice were injected with 50 μl of egg suspension or with an equal volume of PBS per hind footpad.

Abs, Ags, and reagents

FITC- and PE-labeled anti-CD8, PE-labeled CD4, FITC- and PE-labeled anti-IFN-γ, and biotin-labeled anti-IL-2Rβ Abs were purchased from PharMingen (San Diego, CA). Streptavidin-PE was purchased from Jackson ImmunoResearch (West Grove, PA). Rat anti-IL-4R Ab was generously donated by Immunex (Seattle, WA) and used at 2.5 μg/ml. Plate-bound anti-CD3 mAb (PharMingen) was used at 1 μg/well. rIL-6 (Integen, Purchase, NY) was used at 1–10 ng/ml in vitro cultures. Soluble egg Ag (SEA) was prepared as previously described (19, 20) and used at 50 μg/ml. LPS was purchased from Sigma and used at 5 μg/ml. CFSE (5-(and-6)-carboxyfluorescin diacetate, succinimidyl ester) was purchased from Molecular Probes (Eugene, OR) and used at 2.5 μM.

Lymph node (LN) cell preparation and proliferation

Popliteal LN were harvested from egg- or PBS-injected mice, and single cell suspensions prepared using sterile 70-μm nylon sieves (Falcon, Franklin Lakes, NJ) as previously described (21). LN cells were resuspended at 5 × 10^6/ml in complete T cell medium containing DMEM (Life Technologies, Gaithersburg, MD), 10% FCS (Sigma), 100 U/ml penicillin, 100 μg/ml streptomycin, 1 mM L-glutamine (Life Technologies), 10 mM HEPES (Life Technologies), and 5% (v/v) 10 μg/ml M2-ME (Sigma). Cells (10^5/well) were cultured in 96-well flat-bottom plates (Falcon) at 37°C/5% CO2. Culture supernatants were harvested at 24 and 72 h for cytokine analysis. M1 anti-IL-4R Ab was added to LN cultures (2.5 μg/ml) for 48 h to inhibit IL-4 production.

Results

Injection of schistosome eggs results in IL-6 production

To determine whether IL-6 could be playing a role in the differentiation of egg-specific ThP cells into Th2 cells, the production of IL-6 was first analyzed in response to the injection of schistosome eggs into the hind footpads of WT B6/129 mice. Within 24 h of egg injection, IL-6 transcripts were up-regulated at the site of Ag deposition and in the draining popliteal LN (Fig. 1A). Cultured LN cells from egg-injected mice, but not those from PBS-injected animals, produced low levels of IL-6 constitutively (Fig. 1B). Levels of IL-6 were increased over 20-fold by stimulation with either anti-CD3 or LPS (Fig. 1C). Even under these strong polyclonal activation conditions, cells from egg-injected mice made significantly more IL-6 than did those from PBS-injected mice. Interestingly, re-exposure of LN cells from egg-injected mice to SEA in vitro also led to an increase (~2-fold) in IL-6 production (Fig. 1B). These results indicate that eggs induce IL-6 production at a time and site consistent with a role for this cytokine in promoting the differentiation of egg-specific ThP cells into Th2 cells.

Production of IL-4 24 h after egg injection is not eliminated in IL-6−/− mice

To examine the role of IL-6 in promoting early IL-4 production by CD4 T cells, the early (24 h) responses to egg injection of IL-6−/− mice were compared with those of WT mice. LN cells from both egg-injected WT and IL-6−/− mice produced IL-4 within 24 h of stimulation with anti-CD3 in vitro (Fig. 2A). LN cells from mice injected with PBS also produced IL-4 within 24 h but the levels of IL-4 produced were consistently lower than those seen following egg injection (Fig. 2A). Because no significant differences were observed in the levels of IL-4 produced by WT and IL-6−/− LN
cells from egg-injected animals after 24 h of in vitro culture, the initial production of IL-4 in total LN cultures does not depend upon the presence of IL-6.

To determine whether the early egg-induced IL-4 is coming from CD4 T cells and whether IL-6 plays a role in IL-4 production by CD4 T cells after egg injection, CD4 cells were purified from the LN of PBS and egg-injected mice and stimulated for 72 h with anti-CD3. CD4 T cells from PBS-injected mice produced very low levels of IL-4 (Fig. 2B). Egg-injection primed the CD4 T cells to produce substantially higher levels of IL-4, although WT CD4 T cells consistently produced more IL-4 than did the IL-6−/− CD4 T cells (Fig. 2B). The reduced levels of IL-4 measured in the cultures of IL-6−/− CD4 T cells suggest that IL-6 may play a role in the priming of CD4 T cells to produce IL-4 although it is not the sole signal required in the presence of a strong Th2 stimulus (i.e., schistosome eggs).

IL-6−/− mice develop an egg-specific Th2 response following egg injection

Although the absence of IL-6 during CD4 T cell priming did not abolish IL-4 production, the reduced levels produced by the IL-6−/− CD4 T cells could potentially affect the overall development of a Th2 response. Therefore, the role of IL-6 in the development of a Th2 response was investigated by isolating LN cells 7–8 days after egg injection. RT-PCR of RNA isolated from LN from IL-6−/− and WT mice revealed that the levels of IL-4 transcripts were equivalent in the two mouse strains (Fig. 3A). Only low levels of IL-4 transcripts were detected in the LN of PBS-injected mice at this time (Fig. 3A). In vitro stimulation of the LN cells with Ag supported this finding as no significant difference was detected in the levels of IL-4 in 72 h supernatants of the LN cultures from IL-6−/− and WT mice (Fig. 3B). In addition, both WT and IL-6−/− mice developed a Th2 response as demonstrated by the production of IL-4, IL-5, and IL-10 (Fig. 3, B–D) and by negligible levels of IFN-γ (Fig. 3F). IL-2 was also measured in 24-h culture supernatants from both mouse strains (Fig. 3E). Notably, IL-10 and IL-2 levels were lower in the supernatants of cells from egg-injected IL-6−/− mice than in those from WT mice (Fig. 3, D and E). No cytokines were found in the supernatants of LN cells from PBS-injected mice stimulated with SEA (data not shown). Purified CD4 cells from WT and IL-6−/− egg-injected mice displayed a similar cytokine profile after stimulation with anti-CD3 (Fig. 4, A–D), although the disparity in levels of cytokines produced between IL-6−/− and WT mice was less apparent than for the whole LN cell cultures. These results strongly suggest that the absence of IL-6 does not prevent the development of a Th2 cytokine response to schistosome eggs.

Egg-specific Ab response is impaired in IL-6−/− mice

Because the Ab isotypes are also indicative of Th response development and IL-6 is known to play an important role in B cell
differentiation and Ab production (11, 27), the Ab response to egg Ag was studied 7 days after egg injection. SEA-specific Ab in plasma were detected using ELISAs. As shown in Fig. 5, plasma from WT mice contains SEA-specific Ab in which both IgG1 and IgG2a isotypes can be detected, albeit at low levels. Total Ig and IgG2a were considerably decreased in the IL-6−/− mice, with the most striking difference being the total absence of IgG1. These results demonstrate an impairment in the production of both Th1 (IgG2a) and Th2 (IgG1)-associated Ab isotypes. Histological examination of the LN taken 7 days after egg injection revealed an impaired germinal center formation in the IL-6−/− mice compared with the WT mice (data not shown) as was expected given previous reports (27). Therefore, despite the similar Th2 response development in the IL-6−/− mice compared with WT mice, Ab production of both Th1 and Th2-associated isotypes and germinal center formation is impaired, supporting the finding that in the absence of IL-6, B cell development and differentiation is affected.

In vitro proliferation is severely reduced in LN cells from egg-injected IL-6−/− mice

Despite the lack of effect of the absence of IL-6 on Th2 cytokine production, its marked effect on B cell responses prompted the investigation into other known functions of IL-6 which could modify response development after egg injection. In addition to its role in T and B cell differentiation, IL-6 has been shown to promote proliferation of spleen cells in vitro (28). The role of IL-6 in proliferation was clearly demonstrated by visual assessment of the culture wells after 48 h. WT LN cells from mice injected with eggs 7 days previously had more pronounced blast and cluster formation when stimulated with anti-CD3 (Fig. 6A) than did cells from IL-6−/− mice (Fig. 6B). Comparable results were seen on day 1 after egg injection (data not shown). Furthermore, the proliferation of the IL-6−/− LN cells as measured by [3H]thymidine incorporation was also severely reduced compared with WT (Fig. 6C).

**FIGURE 3.** Both WT and IL-6−/− mice develop egg-specific Th2 responses 7 days after egg injection. A, Similar levels of IL-4 transcripts are present in LN of WT and IL-6−/− mice 7 days after egg injection. Total mRNA was isolated from LN and samples were equalized using competitive PCR for HPRT transcripts. B–F, Cytokine levels in supernatants of LN cells from WT and IL-6−/− mice cultured for 24 h (E) or 72 h (B–D and F) with SEA. Cytokine levels were measured by ELISA. Shown for B–F are the means and SEM of three experiments.

**FIGURE 4.** Both WT and IL-6−/− mice develop type 2 CD4 T cells 7 days after egg injection. Cytokine levels of IL-4 (A), IL-5 (B), IL-10 (C), and IL-2 (D) in cultures of purified CD4 T cells from LN of WT and IL-6−/− mice after stimulation for 72 h with anti-CD3. Cytokine levels were measured by ELISA. Shown are the means and SEM of triplicate wells from one of three similar experiments.
Similar results were found when proliferation was measured by flow cytometry of cells labeled with CFSE (Fig. 7). CFSE is a fluorescent cytoplasmic tag which becomes evenly distributed between daughter cells after mitosis and thus, reduction in cellular fluorescence correlates with proliferation. Addition of rIL-6 to cultures stimulated with anti-CD3 enhanced the proliferation of LN cells from WT and IL-6<sup>-/-</sup> PBS (data not shown) and egg-injected mice (Fig. 7), although it did not restore proliferation of the IL-6<sup>-/-</sup> cultures to WT levels. Decreased proliferation was also seen with Ag-specific stimulation (data not shown). The difference in proliferative responses of WT versus IL-6<sup>-/-</sup> LN cells was not due to a difference in cell types present in the LN at day 1 or 7 (Table I) because the small differences in cellular composition could not account for the dramatic difference in proliferation. Interestingly, although the LN cells from the WT PBS-injected mice showed 5-fold greater proliferation on day 1, by day 7 the proliferation had dropped to the level of the IL-6<sup>-/-</sup> LN cells (Fig. 6C). This result suggests that on day 1 the PBS-injected mice are nonspecifically activated by the injection, but by day 7 the activating stimulus has dissipated. Overall, these findings indicate that after activation of LN cells either through introduction of schistosome eggs or immediately after a trauma induced by injection, the proliferation of the IL-6<sup>-/-</sup> cells in vitro is seriously impaired and this proliferative defect can be partially corrected by the addition of rIL-6.

To determine whether the proliferative defect in the IL-6<sup>-/-</sup> LN cultures is specific to one cell type, the proliferation of CD4 and CD8 T cells and B cells during in vitro culture with anti-CD3 stimulation and labeled with CFSE was examined using flow cytometry. As shown in Table II the percentage of proliferating CD4 and CD8 T cells and B lymphocytes from IL-6<sup>-/-</sup> mice injected with eggs 7 days previously was significantly reduced compared with cells from WT mice. Similar results were seen with the T and B cells from PBS-injected mice (data not shown). The addition of rIL-6 to the LN cultures from egg-injected IL-6<sup>-/-</sup> mice primarily enhanced proliferation of CD8 T cells and, to a lesser extent, CD4 T cells (Table II), indicating that exogenous IL-6 can partially overcome the proliferative defect of IL-6<sup>-/-</sup> T cells. Although addition of rIL-6 had only a subtle effect on CD4 proliferation, it had a more pronounced effect on the percentage of CD4 cells in the LN culture that may reflect a decrease in apoptosis rather than an increase in proliferation. This finding is consistent with recent findings that IL-6 prevents apoptosis of CD4 T cells in vitro by maintaining levels of bcl-2 (29). In contrast to its effect on T cells, addition of rIL-6 did not enhance IL-6<sup>-/-</sup> B cell proliferation in vitro.

**FIGURE 5.** SEA-specific Ab production is impaired in IL-6<sup>-/-</sup> mice. Plasma samples were collected and pooled from WT and IL-6<sup>-/-</sup> mice 7 days after egg or PBS injection. Isotype-specific anti-SEA Ab levels were measured by ELISA and expressed as the OD<sub>405</sub>.

**FIGURE 6.** In vitro proliferation of IL-6<sup>-/-</sup> LN cells is impaired. WT (A) and IL-6<sup>-/-</sup> (B) LN cells were isolated 7 days after egg injection and stimulated for 48 h with anti-CD3. C. Proliferation of total LN cells isolated 1 day or 8 days after egg or PBS injection and stimulated for 72 h with anti-CD3. The results expressed as cpm, and shown are the means and SEM of duplicate wells from one of three similar experiments.
reactions. LN cells were labeled with CFSE and stimulated for 72 h 

in vitro nor did it increase the percentage of B cells in the LN cul-
tures, suggesting that the problem in IL-6−/− B cell proliferation may stem from a defect in differentiation in vivo. This conclusion is further supported by evidence that IL-6 enhances proliferation of only highly differentiated B cells (9). Together these results reveal a role for IL-6 in lymphocyte proliferation which is all the more striking given the similar cytokine profiles.

**FIGURE 7.** In vitro proliferation of IL-6−/− LN (bottom) is impaired compared with WT (top). A and B. Addition of rIL-6 (10 ng/ml) enhances proliferation in cultures of WT and IL-6−/− LN cells isolated 8 days after egg injection. LN cells were labeled with CFSE and stimulated for 72 h with anti-CD3 + rIL-6 and analyzed by flow cytometry. Shown are the results from one of two similar experiments.

**Table I.** Cellular composition of popliteal LN

<table>
<thead>
<tr>
<th>Mouse Type</th>
<th>% CD4</th>
<th>% CD8</th>
<th>% B220</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT PBS</td>
<td>42.1 ± 1.9*</td>
<td>27.4 ± 2.7</td>
<td>24.8 ± 2.5</td>
</tr>
<tr>
<td>IL-6−/− PBS</td>
<td>34.3 ± 0.9*</td>
<td>27.1 ± 3.7</td>
<td>33.3 ± 3.3</td>
</tr>
<tr>
<td>WT egg, day 1</td>
<td>32.7 ± 0.5*</td>
<td>28.9 ± 0.9**</td>
<td>27.3 ± 5.0</td>
</tr>
<tr>
<td>IL-6−/− egg, day 1</td>
<td>22.2 ± 0.7**</td>
<td>20.9 ± 1.5**</td>
<td>40.7 ± 5.8</td>
</tr>
<tr>
<td>WT egg, day 7</td>
<td>30.6 ± 2.9</td>
<td>17.9 ± 1.1</td>
<td>51.2</td>
</tr>
<tr>
<td>IL-6−/− egg, day 7</td>
<td>23.4 ± 0.9</td>
<td>12.7 ± 2.3</td>
<td>60.3</td>
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</table>

* p < 0.01 WT vs IL-6−/−.

**Table II.** *In vitro proliferation of LN cell subpopulations*

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Treatment</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
<th>B220⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% total</td>
<td>% proliferating</td>
<td>% total</td>
</tr>
<tr>
<td>WT egg</td>
<td>Anti-CD3</td>
<td>30</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anti-CD3 + IL-6</td>
<td>39</td>
<td>85</td>
<td>30</td>
</tr>
<tr>
<td>IL-6−/− egg</td>
<td>Anti-CD3</td>
<td>20</td>
<td>59</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Anti-CD3 + IL-6</td>
<td>26</td>
<td>62</td>
<td>26</td>
</tr>
</tbody>
</table>

* The % total = percentage of total cells that stained positive (+) after a 72-h in vitro culture. The % proliferating = percentage of positive (+) cells that had proliferated after a 72-h in vitro culture.

**IL-2R expression is reduced on IL-6−/− LN cells and can be restored by the addition of IL-6**

Since IL-2 is a well-characterized growth factor, a possible explanation for the reduced proliferation of the IL-6−/− cells in culture could be reduced production of IL-2. Following anti-CD3 stimulation for 24 h, there are slightly higher levels of IL-2 in culture supernatants of LN cells from WT than IL-6−/− mice that had been injected with eggs 7 days previously (Fig. 8A). However, after 72 h of in vitro stimulation, high levels of IL-2 were detected in the IL-6−/− cultures, whereas no IL-2 was found in the WT LN cultures (Fig. 8B; please note the difference in scales). Comparable results were found using LN cells isolated day 1 after egg injection (data not shown). Addition of rIL-6 to the LN cultures stimulated with anti-CD3 resulted in a dramatic decrease in the levels of IL-2 detected in the WT and IL-6−/− culture supernatants after 24 or 72 h (Fig. 8A and B). Similar results were seen with LN cells from either PBS or egg-injected mice (data not shown). The increased levels of IL-2 in the culture supernatants of the IL-6−/− mice point toward a reduced utilization of IL-2 as the cause of the proliferative defect. Furthermore, the reduced levels of IL-2 detected in culture supernatants after the addition of rIL-6 suggests that IL-6 promotes an increased utilization of IL-2, which in turn enhances the proliferative responses in both IL-6−/− and WT mice.

To determine whether reduced expression of the IL-2R could be responsible for the reduced utilization of IL-2 and impaired proliferation, LN cells from IL-6−/− mice injected with eggs 7 days previously were stimulated for 72 h with anti-CD3 and compared with similarly treated WT cells for IL-2R expression (Fig. 9). Culture with medium alone resulted in a low level of IL-2R expression in both the WT and IL-6−/− cultures whereas addition of anti-CD3 markedly up-regulated the levels on both WT and IL-6−/− cells (Fig. 9). As hypothesized, a lower percentage of IL-6−/− cells expressed the IL-2R at a lower level compared with WT cells. This reduced expression on IL-6−/− cells could be
Discussion

We have been using schistosome eggs, which are strong inducers of Th2 responses, as a model Ag with which to investigate the early in vivo events that promote the development of Ag-specific Th2 and B cell responses. Exposure to IL-4 at or around the time of initial activation plays a central role in promoting naive ThP cells to differentiate into Th2 cells (1). This early IL-4 may be derived from the ThP cell itself or another cell type (e.g., eosinophils, NK1.1+ CD4 T cells). While we and others have shown that cells other than the ThP cell can respond rapidly to Th2 inducing stimuli by making IL-4 (3–7), there is little evidence that this IL-4 is essential for Th2 response development (2). Although this would seem to focus attention on the ThP cell itself as the source of early IL-4, the issue has been confounded by the fact that in most strains of mice ThP cells have been reported to make very little or no IL-4 (30, 31). The discovery that IL-6, a cytokine made in quantity by macrophages and other cell types involved in the innate response, could up-regulate IL-4 production by ThP cells (8), provided a possible explanation for how ThP cells might be able to produce sufficient IL-4 to act as an autocrine differentiation factor. This finding also suggested that IL-6 could be crucial for Th2 response development. We have examined these issues using IL-6−/− mice immunized with schistosome eggs.

For IL-6 to play a role in early IL-4 production it must itself be produced rapidly following egg injection. Our studies confirm that this is the case, with significant levels of IL-6 being produced by LN cells recovered at 24 h postegg, though not PBS, injection. At present we do not know which cells are secreting this IL-6 nor what the stimulus for production is. As in previous studies, we were able to detect early IL-4 production following egg injection, though for the first time we show that at least part of the IL-4 is being produced by CD4 cells. It is our assumption that the cells producing this IL-4 are schistosome egg Ag-specific ThP cells responding for the first time and making IL-4, although this remains to be shown. Regardless, egg injection into WT and IL-6−/− mice results in the activation and/or recruitment of CD4 cells, not present in PBS-injected mice, that are able to make IL-4 when stimulated in vitro. This population of CD4 cells from IL-6−/− mice was found to produce less IL-4 than that from WT mice. We are not clear whether this is due to failure of the IL-4-producing cells to expand in vitro (see below) or to the absence of a more upstream effect of IL-6 on promoting IL-4 production, which would be consistent with the published report that IL-6 promotes IL-4 production by ThP cells (8). Nevertheless, the slight early differences in IL-4 production did not have a long-term effect on the outcome of the Th2 response as, in the absence of IL-6, the development following exposure to schistosome eggs of Th cells capable of making signature type 2 cytokines such as IL-4 and IL-5, was not impaired.

Although the ability to produce type 2 cytokines was unaffected in IL-6−/− mice, we found that in the absence of IL-6 proliferation of LN cells was dramatically impaired and that this defect in proliferation encompassed CD4 and CD8 T cells as well as B cells. The connection between IL-6 and proliferation of T cells, B cells, and other cell types has been described before (9, 28, 32), but this is the first report of the in vitro impairment of proliferation in IL-6−/− mice as well as an associated in vitro and in vivo defect in IL-2R expression. We believe this defect in IL-2R expression accounts for the reduced proliferative response because addition of exogenous IL-6 enhanced proliferation of IL-6−/− LN and T cells and caused an up-regulation in IL-2R expression. Interestingly, addition of exogenous IL-6 did not augment proliferation of B cells from IL-6−/− mice. Because IL-6 is a late acting differentiation factor for B cells, the lack of effect of rIL-6 on IL-6−/− B cells may reflect an in vivo defect in B cell development (see below). These results complement recent observations that anti-IL-6 Ab blocks proliferation of WT cells (28), that Ab to IL-2 or the IL-2R blocked IL-6-induced T cell proliferation (32), and that IL-2R expression could be induced on T cell lines by IL-6 (33). Taken together, our data reinforce the view that IL-6, IL-2R expression and T cell proliferation are interlinked.

The relationship between IL-6 and proliferation is a possible explanation for the effects of IL-6 on Th2 differentiation in vitro. Addition of IL-6 may promote the production of IL-2 and, consequently, directly or indirectly influence IL-4 production by naive ThP cells. In studies by Seder (34), it was shown that IL-2 was required for IL-4 production and in vitro proliferation of naive CD4 T cells. The necessity for IL-2 production and proliferation
before IL-4 production can occur may thus define the relationship between IL-4 and IL-6. However, these results do not exclude the role of other cytokines in promoting proliferation (e.g., IL-1β) nor can they yet be extended to the in vivo situation. Therefore, while this proliferative defect in the IL-6−/− mice manifests itself in vitro, it is not clear that it occurs in vivo nor does it seem to affect the course of Th2 development in the mouse.

Although the lack of IL-6 failed to affect the development of Th cells capable of making the type 2 cytokines, it had a marked effect on B cell responses following immunization with decreased levels of total Ig and IgG2a as well as the complete absence of IgG1 compared with egg-injected WT mice. IL-6−/− mice have been shown to have defective germinal center formation characterized by diffusely structured follicles which lack the well-defined surrounding T cell areas (27). In previous studies this was linked to a deficit in T cell-dependent Ab production (27, 35) since Kopf et al. (27) reported defects in total IgG and IgG2a levels but found no significant differences on IgM and IgG1 production. The differences in our results may stem from the use of different Ag or differences in the timing of the response, for our studies are focused on early time points. It is possible that if the B cell response had been allowed to proceed longer, differences in IgG1 levels would have been less marked. Another indication of an in vivo B cell defect was the impaired in vitro B cell proliferation that could not be enhanced by the addition of rIL-6. Because IL-6 is a late stage B cell differentiation factor, a defect in vivo B cell differentiation could result in the isolation and culture of undifferentiated B cells that are unable to proliferate in response to exogenous IL-6. Furthermore, since Th cells from immunized IL-6−/− mice are as capable as WT Th2 cells of producing cytokines, the

**FIGURE 9.** IL-2R expression is reduced on LN cells from IL-6−/− versus WT mice injected with eggs 7 days previously and cultured for 72 h with medium alone, anti-CD3, or anti-CD3+ rIL-6. LN cells were incubated with an isotype control Ab or with anti-IL-2R mAb. The results are expressed both as the percentage of positive cells and the MFI of the anti-IL-2R stained cells.
defect in the B cell response is unlikely to stem from a lack of cytokine-mediated help. Other possible explanations for the impaired B cell response include a direct effect of IL-6 on B cells or the failure of T cells to express appropriate cell surface costimulatory molecules involved in B cell help (27, 35, 36). Regardless, our results point to a defect early in the development of the B cell response in IL-6−/− mice responding to schistosome eggs.

By using IL-6−/− mice, we have been able to better define the role of IL-6 in the promotion of T and B cell responses after the injection of a Th2 response-inducing Ag. Although egg injection resulted in the induction of IL-6 and Th2 development in WT mice, the absence of IL-6 did not prevent Th2 development in IL-6−/− animals. Despite the lack of effect of the absence of IL-6 on Th responses per se, B cell response development and LN cell proliferation were seriously impaired in the IL-6−/− mice. The defect in proliferation correlated with decreased IL-2R expression, whether proliferation and/or IL-2R expression is responsible for the impaired B cell responses in vivo.

References


