Distinct requirements for IL-6 in polyclonal and specific Ig production induced by microorganisms

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Abstract

The role of IL-6 in Ig production induced in the mouse by lactate dehydrogenase-elevating virus (LDV), Toxoplasma gondii or lipopolysaccharide (LPS) was assessed. Following infection with LDV, a strong activator of B cells, an early and transient IL-6 production was observed, that originated predominantly from macrophages. Whereas LDV-induced B lymphocyte proliferation appeared independent of IL-6, mice deficient for this cytokine showed a marked reduction in their total T-dependent IgG2a production when compared to their normal counterparts. By contrast, specific responses directed against either LDV or non-viral antigens administered at the time of infection were not decreased in the absence of IL-6. Similarly, polyclonal, but not anti-parasite IgG2a production triggered by T. gondii infection was strongly dependent on the presence of IL-6. Finally, T-independent total IgG3 secretion triggered by LPS was also markedly reduced in IL-6-deficient mice. These results suggest that IL-6 plays a major role in T-dependent and T-independent polyclonal Ig production following B lymphocyte activation by viruses, and parasites, but not in specific antibody responses induced by the same microorganisms.

Introduction

Infection of mice by some microorganisms including viruses, parasites and bacteria, or inoculation of products such as lipopolysaccharide (LPS), induce, in addition to secretion of specific antibodies, a strong B lymphocyte polyclonal activation resulting in hypergammaglobulinemia (1–4). The isotypic distribution of these Ig depends on the stimulus involved: LPS induces a response that is dominated by IgM and IgG3 (4–6); responses induced by parasites such as Plasmodium chabaudi, Trypanosoma cruzi and Toxoplasma gondii are restricted to the IgG2a subclass (7–10); a similar IgG2a preponderance has been reported after infection with viruses like lactate dehydrogenase-elevating virus (LDV), mouse hepatitis virus, murine adenovirus, lymphocytic choriomeningitis virus and murine cytomegalovirus (1,6,11–14). The enhanced production of natural antibodies resulting from such B cell polyclonal activation may play an important role in the defense against infections, especially at the early times after invasion of the host by viruses or bacteria, when specific responses have not yet matured (15). However, the mechanisms leading to this type of immune response are not fully understood. In many cases, a T lymphocyte-independent proliferation of B cells (1,14,16) results probably from a direct interaction of a microorganism product with some receptor expressed on these cells (17–20). In contrast, Ig switch and secretion by these activated B lymphocytes are more likely regulated by Th cell-dependent mechanisms involving interaction with cytokines (1,13,21–24).

Among the cytokines capable to stimulate B lymphocytes, it has been reported that IL-6, in synergy with IL-1, induces B cell proliferation and secretion of large amounts of IgM by those cells (25). Moreover, the production of other Ig isotypes, such as IgA and IgG, by B lymphocytes already committed to their secretion is enhanced by IL-6 (26,27). In addition, IL-6 is a potent in vitro and in vivo growth factor for murine plasmacytomas (28–30). Therefore, it is plausible to hypothesize that IL-6 could play a role in microorganism-
triggered Ig production, including that following B lymphocyte polyclonal activation, especially since the secretion of this molecule has been shown to be induced by both LPS and parasitic and viral infection (31–38). Our results indicate that, at least in some mouse strains, IL-6 is indeed required for hypergammaglobulinemia induced by microorganisms and derived products, but not for the secretion of specific antibodies, suggesting that specific or polyclonal B lymphocyte activations are differentially regulated by this cytokine.

### Methods

#### Mice

Isolator-reared 129/Sv female mice and SPF BALB/c mice were produced at the Ludwig Institute for Cancer Research by Dr G. Warnier and used when 8–12 weeks old. B6,129-IL6tm1Kopf mice and their controls, B6,129F2/J animals (39), were obtained from the Jackson Laboratory (Bar Harbor, ME).

#### Virus, parasite, LPS and antigen

*In vivo* infection was performed by i.p. injection of ~2 × 10^7 50% infectious doses (ID50) of LDV (Riley strain; ATCC, Rockville, MD) (6). Mice were infected with the weakly virulent Beverley strain of *T. gondii* by i.p. inoculation with 5 cyst parasites, as described previously (10). LPS from *Escherichia coli* (O55:B5; Difco, Detroit, MI) was injected i.p. (25 μg in 500 μl saline per mouse). Immunization with keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, CA) was performed by i.p. injection of 100 μg antigen in 500 μl saline.

#### Antibody

Anti-CD4 mAb GK1.5 (40) was made available by Dr F. W. Fitch (Chicago) and obtained through the courtesy of Dr H. R. MacDonald (Epalinges sur Lausanne, Switzerland).

#### Spleen cell cultures

As described previously (6), 25 × 10^6 spleen cells were cultured in 5 ml Iscove’s medium containing 10% FCS and supplemented with 0.24 mM L-asparagine, 0.55 mM L-arginine, 1.5 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 0.1 mM hypoxanthine and 0.016 mM thymidine. Cells were counted 4 days later by hexosaminidase determination (43). Results, expressed in U/ml, were defined as the concentration producing half-maximal growth of the cells.

#### IL-6 assay

IL-6 was assayed as described in (42) by incubation of serial sample dilutions with the mouse IL-6-dependent B cell hybridoma 7TD1 (2000 cells/microwell) in 0.2 ml Iscove’s medium containing 10% FSC, and supplemented with 0.24 mM L-asparagine, 0.55 mM L-arginine, 1.5 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 0.1 mM hypoxanthine and 0.016 mM thymidine. Cells were counted 4 days later by hexosaminidase determination (43). Results, expressed in U/ml, were defined as the concentration producing half-maximal growth of the cells.

#### Antibody determination

Total IgG subclasses were determined by direct ELISA, as described previously (1). The binding of IgG subclasses to insolubilized mouse IgG isotype-specific rabbit antibody or to insolubilized mouse IgG isotype-specific rat mAb was measured with peroxidase-labeled anti-mouse IgG (obtained from H. Bazin, Brussels) or donkey antibody. Standards were mAb of the appropriate isotype. All IgG2a allotypes were recognized by the IgG2a-specific assay.

Specific antibody IgG2a was assayed by ELISA as described previously (10, 24), by using plates coated with appropriate antigens and standard curves of selected anti-DNP mAb.

#### RNA extraction and PCR amplification

Gene expression was analyzed by RT-PCR as described previously (44). Cells were lysed in Trizol reagent (BRL, Gaithersburg, MD). Total RNA was first extracted with chloroform, then precipitated with isopropanol, washed in ethanol and finally resuspended in 50–100 μl water. Oligo(dT)-primed cDNA was prepared from ~5 μg RNA using 200 U MMLV reverse transcriptase (BRL) according to the manufacturer’s instructions. cDNA was amplified by PCR with DyNAzyme DNA polymerase (Finnzymes, Espoo, Finland) for actin and with a Gene Amp kit (Perkin-Elmer Cetus, Norwalk, CT) for IL-6 in a Thermal Reactor (Hybaid, Middlesex, UK). The primers were as follows: actin: AGGCATTGTGATGGACTCC and GCTGGAAGGTGGACAG-TGAG; IL-6: ATGAAGTTCC-CTTCAC.

The post-PCR products were analyzed in 1% agarose gels containing ethidium bromide. Semi-quantitative results were obtained after blotting of the PCR products on Zeta-Probe membranes (BioRad, Hercules, CA) and hybridization overnight at 42°C in Denhardt's solution with internal probes labeled with 32P. The radioactivity was quantitated with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA), and the ratios between IL-6 and actin messages were calculated after subtraction of non-specific background and shown as arbitrary units. The sequence of the probes was: actin: TATGACCTGCCTGACGGA; IL-6: GACCTGTCTATACCA-CCTCAC.

### Results

#### IL-6 secretion in mice infected with LDV

Although it has been shown that many viruses trigger IL-6 secretion, little is known so far on the production of this...
cytokine after LDV infection. To analyze the role of IL-6 in LDV-induced Ig production, we first determined whether this virus triggered secretion of the cytokine. Thus, IL-6 was bioassayed in the serum of 129/Sv animals at different times post-infection (p.i.). As shown in Fig. 1(A) for one experiment among four performed, a transient peak of serum IL-6 was detected during the first day p.i. At later times p.i., no IL-6 was found by this method (data not shown). These results were confirmed by RT-PCR analysis of IL-6 message expression in spleen and peritoneal cells from 129/Sv mice. A strong, but transient IL-6 message was induced by the virus (Fig. 1B, shown for one of three experiments performed). This IL-6 expression peaked at 24 h p.i. in spleen cells while it was already nearly at its maximum at 6 h p.i. in peritoneal cells. At 36 h p.i., the IL-6 message returned to nearly normal levels both in spleen and peritoneal cells. Similar kinetics were observed in spleen and peritoneal cells of LDV-infected CBA mice (data not shown).

IL-6 can be produced by different cell populations, including macrophages and T lymphocytes. To determine the role of the latter cells in LDV-induced IL-6 secretion, we treated mice with anti-CD4 GK1.5 mAb, since this treatment had been previously found to deplete T lymphocytes in vivo (40,45). No modification in IL-6 secretion was observed after this treatment (Table 1, results representative of two experiments). Moreover, BALB/c nu/nu and BALB/cBy-SCID mice produced as much IL-6 after LDV infection as their normal BALB/c counterparts (data not shown). The cellular origin of LDV-induced IL-6 was also analyzed by RT-PCR, after purification of spleen cell subpopulations. At 12 h p.i., a strong IL-6 message was detected in MAC-1-enriched cells, but not in B lymphocytes (shown in Fig. 2 for two independent experi-
Ig regulation by IL-6

Role of IL-6 in B lymphocyte responses triggered by LDV infection

LDV infection induces a polyclonal B lymphocyte activation characterized by both a T-independent cell proliferation and a T-dependent Ig secretion restricted to the IgG2a subclass (1,24). The role of IL-6 in these effects was analyzed in B6129F2/J (IL-6+/+) or B6.129-IL6tm1Kopf (IL-6–/–) mice. LDV-induced B lymphocyte proliferation, measured by thymidine incorporation, was similar in IL-6-deficient and normal animals (data not shown). In contrast, a strong reduction of total serum IgG2a levels following LDV infection was observed in IL-6-deficient mice when compared to normal infected animals (Fig. 3A). Although LDV-induced IgG2b secretion seemed slightly increased in IL-6-deficient mice when compared to normal infected animals, the difference was not significant (P > 0.05). No IL-6-related difference was observed for IgG3. Finally, basal IgG1 levels were slightly higher in IL-6-deficient mice than in normal animals (P = 0.03 and ≤0.01 for control and infected mice, respectively). Despite this difference, no increase in IgG1 levels followed LDV infection of IL-6-deficient mice. This control of LDV-induced total IgG2a secretion by IL-6 was confirmed by spleen cell cultures. As shown in Fig. 4 for one of two experiments performed, the production of total IgG2a by spleen cells obtained from IL-6-deficient mice 1 week after infection was indeed much lower than that of cells from their normal counterparts.

Like total Ig, specific antibody responses elicited against viral or non-viral antigens in mice infected with LDV and concomitantly immunized with a protein antigen such as KLH are restricted to the IgG2a subclass (11,12). However, contrasting with total IgG2a, the production of specific IgG2a anti-LDV antibodies was not significantly decreased in animals deficient for IL-6 (Fig. 3B, shown for a typical experiment among three). Similarly, the anti-KLH IgG2a antibody response elicited in LDV-infected mice after immunization with this antigen was not significantly lower in the absence of IL-6 (Table 2, one among two experiments done, P = 0.4 by Mann–Whitney test).

Requirement of IL-6 for T. gondii-induced Ig responses.

Like LDV, the weakly virulent Beverley strain of T. gondii induces an IgG2a-restricted polyclonal activation of B lymphocytes (10) and IL-6 production (37). To assess the role of the cytokine in this polyclonal IgG2a secretion, we infected IL-6-deficient mice with the parasite. As shown in Fig. 5(A) for a typical experiment among four, the rise in total IgG2a serum
Table 2. IL-6-independence of specific antibody responses in mice infected with LDV and immunized with KLH

<table>
<thead>
<tr>
<th>Mice</th>
<th>Anti-KLH IgG2a antibody (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6129F2/J</td>
<td>28 ± 5</td>
</tr>
<tr>
<td>B6,129-IL6tm1Kopf</td>
<td>16 ± 6</td>
</tr>
</tbody>
</table>

*aGroups of three mice.
*bMeasured by ELISA in sera obtained 28 days after LDV infection and immunization with KLH (same animals as in Fig. 3B, means ± SE).

Discussion

In vitro, the ability of IL-6 to enhance Ig secretion by mouse B lymphocytes is well established (26,27). Similarly, an in vivo effect of IL-6 on Ig secretion has been demonstrated with mice overexpressing this cytokine (47). In the present study, analyzing the actual involvement of IL-6 in pathological situations characterized by an enhanced Ig production, we showed that both T-dependent and T-independent hypergammaglobulinemia following B lymphocyte polyclonal activation triggered by microorganisms required the presence of this cytokine. In contrast, specific antibody responses appeared to be IL-6-independent.

Our results indicate that, after LDV infection, a transient production of IL-6 occurred that originated mostly from macrophages rather than from T lymphocytes. It has been suggested previously that LDV does not trigger IL-6 production and
Therefore that LDV-induced immune alterations could not be related to this cytokine (48). However, this analysis of IL-6 production was performed in chronically infected mice, which can easily account for the difference with our results that showed a rapid decrease of the cytokine expression 2 days after infection. On the other hand, our data fit well with other reports of IL-6 secretion by mouse or human macrophages after infection with different viruses, including Newcastle disease virus, respiratory syncytial virus and coxsackievirus (49–51). Together with a previous report of early IL-12 expression following infection (41), our observation indicates that LDV triggers a transient macrophage activation that may initiate a cascade of events responsible for some of the effects of the virus on the immune responses, such as B lymphocyte polyclonal activation. It remains to be determined whether this cytokine production originates only from infected macrophages or involves also non-infected recruited cells.

Different studies have so far analyzed the role of IL-6 in Ig production by using either anti-IL-6 antibodies or mice deficient for this cytokine, often with apparently conflicting results. Only moderate influence on total IgG2a serum levels were observed after treatment with anti-IL-6 or anti-IL-6 receptor antibodies in BALB/c or NZB/W F1 mice, although an inhibition of IgG1 responses was found (52,53). Inhibition of total or specific Ig was reported in B6×129 mice deficient for IL-6 after administration of various stimuli such as ovalbumin, myelin oligodendrocyte glycoprotein peptide, vaccinia virus or murine cytomegalovirus (14,54,55). Interestingly, a strong decrease of IgG2a, IgG2b and IgG3, but not IgG1 antigen-specific antibodies was reported in these animals after immunization with DNP–ovalbumin (56). In contrast, whereas administration of *Schistosoma mansoni* eggs resulted in a decreased specific anti-egg antigen IgG1 and IgG2a antibody secretion in IL-6-deficient mice (57), IgG production by granuloma cells following s.c. infection with the same parasite was not modified in the absence of the cytokine (58). Similarly, a polyclonal IgG2a production induced by gammaherpesvirus 68 appeared to be IL-6-independent in the same B6×129 mice (59). In addition, independent studies performed with IL-6-deficient mice of the 129/Sv genetic background showed, in the absence of the cytokine, an increase of IgG2a antibodies after immunization with ovalbumin and infection with *T. gondii* (60,61). Interestingly, in contrast to the large decrease in total IgG2a production reported here after LDV infection of IL-6-deficient B6×129 mice, in C57BL/6 animals, LDV-induced total IgG2a production was IL-6-independent (data not shown), which fits well with a similar difference between mouse strains reported after ovalbumin immunization (56). These results indicate that the effect of IL-6 on B lymphocytes may vary from one mouse strain to another, and thus that the genetic background must be taken into consideration when analyzing the effect of cytokines on Ig secretion.

At this point, the mechanisms by which IL-6 enhances Ig production *in vivo* are not completely understood, although it has been shown that the cytokine may directly increase B cell growth and differentiation *in vitro*, especially in conjunction with IL-1 (25). Apparently conflicting results have been reported on the ability of IL-6 to affect T<sub>H</sub> lymphocyte differentiation (57,62). However, because T-independent LPS-induced IgG3 production was affected by the absence of IL-6 as well as T-dependent responses triggered by virus and parasite, it seems reasonable to postulate that this effect of the cytokine on B cells does not require the presence of T lymphocytes. Although in some models IL-6 was able to induce IgG1 responses (47), this isotype, whereas secreted at rather low levels in our models, was not decreased in the absence of IL-6, a finding reported by other authors as well (56), and was even higher in control IL-6-deficient B6×129 mice than in their normal counterparts. In addition to a mere stimulation of B cell Ig secretion, IL-6 might thus be able to modulate Ig isotypic distribution, in favour to IgG2a, IgG2b and/or IgG3.

Interestingly, in our models, IL-6 was required for the production of total IgG subclasses, but not of IgG2a antibodies specific for viral or parasite antigens, or for proteins that were administered at the time of infection. In addition, we have recently reported that anti-LDV specific antibody responses were controlled by IFN-γ, but that total Ig production triggered by LDV or *T. gondii* did not require the presence of this cytokine (24) that is produced after infection with both infectious agents (10,63 and manuscripts in preparation). Together, these observations strongly suggest that parasite- or virus-induced total polyclonal IgG and specific antibody secretion originate from B lymphocytes that are differentially regulated by cytokines. It may thus be postulated that two distinct and successive humoral responses are triggered by primary infections: early after invasion of the host, a polyclonal production of IgG2a might enhance the levels of natural antibodies recognizing microorganisms, even with a low affinity, that will help to restrict their proliferation (15). The nature of this polyclonal Ig response remains unsolved. The IL-6-independence of the anti-KLH IgG2a antibody response that developed in mice immunized at the time of infection suggests that this cytokine does not enhance all ongoing immune responses. However, it remains possible that some concomitant responses directed against particular antigens, such as carbohydrates or lipids, and/or originating from specific B cell subpopulations, such as B1 cells, could be increased by the virus through IL-6 secretion. Alternatively, this enhanced antibody production may correspond to the stimulation of long-lived plasma cells already committed to IgG secretion (64,65). Why this response develops so fast may be explained by its control by IL-6, which is secreted by macrophages immediately after infection. Although other mechanisms are certainly also involved, it is possible that increased susceptibility of IL-6-deficient mice to various viruses, bacteria and parasites (39,61,66) is, at least partly, related to an impairment of this early polyclonal B cell response. Following this early secretion of total Ig, a more specific antibody response, involving longer recruitment of specific antiviral or anti-parasite B lymphocytes, and controlled by IFN-γ, whose secretion requires subsequent activation of different cell populations like NK cells or lymphocytes, but not by IL-6, will then complete and tighten the control of the invading microorganism.

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**Abbreviations**

KLH keyhole limpet hemocyanin  
LDV lactate dehydrogenase-elevating virus  
LPS lipopolysaccharide  
p.i. post-infection

**References**


Ig regulation by IL-6: 1119
Ig regulation by IL-6


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