Review Article

IL-4 from Th2-type cells suppresses induction of delayed-type hypersensitivity elicited shortly after immunization

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Summary The pure delayed-type hypersensitivity reaction obtained in 4-day ovalbumin-sensitized mice after antigen challenge in the footpad was abrogated by transfer of in vitro expanded, antigen-specific lymphoblasts derived from ovalbumin-hyperimmunized donors (high antibody producers), 12 h before immunization. This effect was specific inasmuch as Trypanosoma cruzi-specific blasts derived from Tc-Ag-hyperimmunized mice did not inhibit delayed-type hypersensitivity in ovalbumin-immunized recipients. The ovalbumin-specific blasts displayed a Th2 cytokine profile, secreting IL-4 and IL-10 upon restimulation in vitro with ovalbumin, but not IFN-γ or IL-2. In addition, recipients of such cells produced much more IgG1 and IgE antibodies. When the frequency of T-cell blasts was enriched among these cells, transfer of four million cells was enough to prevent the induction of delayed-type hypersensitivity. Neutralization of IL-4 alone just before cell transfer not only restored the delayed-type hypersensitivity reaction, but also maintained it in a plateau for at least 72 h after challenge. Recipients treated in this way also showed a shift back towards a Th1 phenotype, indicated by the increase in IL-2, IFN-γ and IL-12 synthesis. No synergistic action was observed when IL-4 and IL-10 were concomitantly neutralized. These results indicate that activation of Ag-specific Th2 cells early in the course of the immune response to a protein antigen provides an immunological environment rich in IL-4, thus leading to the inhibition of cell-mediated immunity.

Key words: delayed-type hypersensitivity, Th2 cells, IL-4.

Introduction

The downregulation of Th1 by Th2-mediated responses in vivo has been documented in numerous reports. In many of them, the responses were directed towards stage-specific antigens from the same parasite, different parasite antigens or parasite antigen and unrelated antigen. In others, the Th2 cells were induced by IL-4 therapy. Therefore, we deemed it important to investigate the interaction between Th1 and Th2 cells stimulated in vivo by a single defined antigen during the time course of a conventional immune response. For this, we used a protocol of subcutaneous immunization of mice with ovalbumin (OVA) in complete Freund’s adjuvant (CFA), in which cell-mediated and humoral immunity could both be detected but at different time-points. A pure delayed-type hypersensitivity (DTH) reaction was obtained in the footpad after challenge with aggregated antigen on day four of immunization, with no detectable circulating antibody. The histopathology of this reaction with a prominent mononuclear cell infiltrate and its time course (peak at 48 h) resembled the tuberculin-type reaction. On day eight, when antibodies were detected in plasma, the characteristics and time course of DTH changed, suggesting the modulation of Th1 by the Th2 emerging response. In order to study the mechanism responsible for such modulation, antibodies were injected before immunization in a group of mice that was challenged four days later, but they did not have any effect upon the induction or type of DTH. However, transfer of spleen cells from high antibody producers (those that do not develop DTH reactions) before immunization abolished the development of DTH in recipient mice. Such an effect seemed to be mediated by Th2 cells, since OVA-specific, T-enriched spleen cells were more suppressive than the same number of whole spleen cells and mice that received the former cells showed a different cytokine profile, synthesizing more IL-4 and IL-10 and less IFN-γ.

According to Rizzo et al., antigen-specific Th1 and Th2 clones, generated in vitro and adoptively transferred to nude mice, interact in vivo to determine the outcome of antibody and DTH responses. The result of this interaction is a decrease in the magnitude of DTH as compared to the response obtained when only a Th1 clone is present. IL-4 and IL-10 are the Th2-derived cytokines involved in the inhibition of the inductive and effector phases of DTH, as shown in immunization or infection protocols. In addition, IL-10 inhibited vascular leakage and swelling during a DTH reaction induced by injection of Th1 clones into mouse footpads. The effect of either type of cytokine, or a simultaneous effect of both cytokines, however, varies according to the experimental model and is not yet well established.

In this work, we transferred in vitro-expanded, antigen-specific lymphoblasts, or enriched T-cell blasts obtained from high antibody producers, into recipient mice that were subsequently immunized and challenged after four days. IL-4, IL-10 or both cytokines were neutralized in such recipients by the administration of cytokine-specific monoclonal antibodies, in order to determine the role of each cytokine in the mechanism mediated by these cells that suppress the induction of DTH.
IL-4 suppresses induction of early DTH

Methods

Animals

Female DBA/2 mice were obtained from a colony at the Instituto de Ciências Biomédicas (Universidade de São Paulo, São Paulo, Brazil) and used for immunization and for transfer experiments at 7–9 weeks of age. Male and female Wistar rats were obtained from a colony at the Instituto de Ciências Biomédicas (Universidade de São Paulo, São Paulo, Brazil) and used for passive cutaneous anaphylactic (PCA) reactions. The experimental protocols were approved by the Biomedical Sciences Institute/USP Ethical Committee for Animal Research.

Antigens and antibodies

Grade II and V OVA and CFA were obtained from Sigma Chemical (St Louis, MO, USA). Aluminium hydroxide gel (Aldrox; Fontoura-Wyth, São Paulo, Brazil) was used as adjuvant for immunization of hyperimmune donors. Concanavalin A (Con A; Sigma) and anti-CD3 mAb were used for in vitro stimulation of T cells. Trypanosoma cruzi (Y strain) tissue-culture trypomastigote antigen (Tc-Ag) was prepared from parasites harvested from infected monolayers of LLC-MK cells (American Type Culture Collection, ATCC, CCL7) and submitted to 10 cycles of freezing and thawing, as previously described. Aliquots were stored at –20°C until used. All the mAb for cytokine assays were purified by protein G-Sepharose chromatography from hybridoma cell culture supernatants and biotinylated, as needed, in our laboratory. Biotin-labelled SXC-1 (anti-IL-10) as well as hybridomas and recombinant standard cytokines were a gift from Dr R. L. Coffman (DNAX Research Institute, Palo Alto, CA, USA).

Immunization protocol and skin testing

Immunization and skin testing of the animals were performed as previously described. Briefly, six mice were injected s.c. with 100 µg of OVA (grade V) emulsified in CFA at the base of the tail. Four days after immunization 30 µL of 2% aggregated OVA (grade II) were injected in one hind footpad and 30 µL of saline in the other. Footpad thickness was measured until 72 h after challenge and the net increase expressed as the arithmetic mean ±SEM of each group. A two-way analysis of variance followed by multiple comparisons using the Tukey method was employed to compare the different groups.

Cytokine assays

For in vitro cytokine measurements, lymphoblasts or lymph node (LN) cells were cultured in 24-well tissue culture plates at a final concentration of 10 or 6 × 10⁴ cells/mL in supplemented Dulbecco’s modified Eagle’s medium and stimulated with OVA (500 µg/mL) or Con A (5 µg/mL). Supernatant fluids were harvested after 24 (10 × 10⁶ cells) or 72 h (6 × 10⁶ cells) and assayed for cytokine content. All the cytokines were measured by specific two-site sandwich ELISA, using the following mAb: (i) JES6-IA 12 and biotinylated JES6–5H4 for IL-2; (ii) XMG 1.2 and biotinylated AN 18 for IFN-γ; (iii) BVD-1D11 and biotinylated BVD6–24G2 for IL-4; and (iv) C252–2A5 and biotinylated SXC-1 for IL-10. Binding of biotinylated monoclonal antibodies was detected using streptavidin-biotinylated horseradish peroxidase complex (Amersham International, Amersham, UK) and 2′-2′-azino-bis (3-ethylbenz-thiazoline-6-sulphonic acid) (Sigma) as substrate diluted in 0.1 M citrate buffer containing hydrogen peroxide. Samples were quantified by comparison with standard curves of recombinant mouse cytokines.

Titrations of antibody isotypes by ELISA

Four-day OVA-immunized recipient mice, transferred with blast cells before immunization, were bled three days after antigen challenge in the footpad. IgG1 and IgG2a antibodies were measured by sandwich ELISA using antigen-coated 96-well plates and biotinylated goat antirabbit isospecific antisera. The reactions were developed with ExtrAvidin®-peroxidase conjugate (Sigma), o-phenylenediamine and H₂O₂, and the plates were read at 450 nm on an automated ELISA reader (MR 5000; Dynatech, Chantilly, VA, USA). The results are reported as the mean absorbance of samples/group (±SEM) at various plasma dilutions. Analysis of variance followed by multiple comparisons using the Tukey test was employed to compare the antibody response among groups.

Titrations of IgE antibodies by PCA

IgE antibodies were measured by passive cutaneous anaphylaxis (PCA) as described by Mota and Wong. IgE reactions were performed in rats, using a sensitization period of 18–24 h and OVA in Evan’s blue solution for challenge. The PCA titre was expressed as the reciprocal of the highest dilution of plasma that gave a lesion of >5 mm in diameter in triplicate tests. Since variation of the PCA titre for the same sample was 2-fold or less, only differences above this value were considered significant.

Hyperimmunization of donor mice

For the induction of high levels of antibodies, DBA/2 mice were immunized s.c. with OVA (50 µg/animal) and aluminium hydroxide (alum; 7.5 mg/animal) as adjuvant in the base of the tail. After 14 and 28 days, the animals were boosted s.c. with 5 µg of OVA. As a control, another group of mice was injected with Tc-Ag (5 × 10⁴ parasites/animal) and same amount of adjuvant and were boosted on days 14 and 28 with Tc-Ag in PBS (5 × 10⁴ parasites/animal).

Obtaining Ag-reactive lymphoblasts

Two weeks after the last booster, the hyperimmune groups were bled and erythrocyte-depleted cell suspensions prepared from their spleens. 5–6 × 10⁷ cells were then incubated in 75 cm² cell culture flasks with OVA or Tc-Ag. An IL-2-rich supernatant (obtained from EL-4 cells cultured with 10 ng/mL of phorbol myristate acetate for 24 h) was added to the cultures after 24 and 72 h (10% v/v). After an additional 72 h, the flask were gently washed and the adherent cells removed with a cell scraper. Large-size low-density cells were isolated by centrifugation (1200 g, 15 min, 22°C) on a discontinuous Percoll gradient (25, 40, 50, and 70%) and collected from the 40–50% and 50–70% interface. The cells were then washed four times and resuspended in culture medium for inoculation in recipient mice before immunization or further stimulation in vitro with OVA, Con A or anti-CD3 mAb for cytokine assays. A feeder of 1 × 10⁷ irradiated (3000 rads) spleen cells was used when the cells were incubated with OVA or Con A. Microplate wells were coated with anti-CD3 mAb overnight before cell incubation.

Enrichment of T-cell blasts

The T-cell blasts obtained from the discontinuous Percoll gradient were further enriched by negative selection, using rat anti-B220 (RA36b2) and anti-Mac1 (M1/70) mAb and a suspension of magnetic beads coated with goat anti-rat IgG antibodies. Labelled cells were removed by exposure to a magnetic field. The T-cell blast-enriched
population was analysed for T cells by flow cytometry (FACscalibur; Becton Dickinson San Jose, CA, USA), using PE-conjugated anti-CD4, FITC-conjugated anti-CD8 and Cy-conjugated anti-B220 mAb, and injected i.v. into recipient mice before immunization. The fraction of large-size/low-density cells originally comprised 22.8% T-cell blasts and 54% B-cell blasts. After the negative selection, the proportion of T-cell blasts increased to 71.8% while that of B-cell blasts was reduced to 1%.

Neutralization of cytokines in vivo
Recipient mice were given two doses of 3 mg/animal of rat anti-IL-4 (11B11) mAb, one day before and two days after cell transfer and immunization, and/or one dose of 2 mg/animal of rat anti-IL-10 (2A5) mAb one day before. The control groups were injected with the same amount of rat IgG.

Results
Suppression of cell-mediated immune response to ovalbumin occurs only in recipients of in vitro expanded lymphoblasts specific to the same antigen
Spleen cells from donor mice hyperimmunized with OVA in alum contain T cells that suppress the induction of pure DTH in 4 day-immunized recipient mice. OVA-specific parameters of the immune response in these donors have been previously described. To expand the population of OVA-specific cells, antigen plus IL-2 were added to cultures of spleen cells from hyperimmune donors and, after six days, large size/low-density cells were isolated on a discontinuous Percoll gradient. In order to compare the effect of lymphoblasts specific to an unrelated antigen, the same protocol was used to expand the spleen cells obtained from a second group of donor mice hyperimmunized with Tc-Ag in alum. The cells obtained from the Percoll gradient were restimulated i.v. into recipients (1.2 × 10^7 cells/animal) 12 h before immunization with OVA in CFA. As shown in Figure 1, recipients of OVA-specific cells had suppressed DTH reactions when challenged with aggregated OVA in the footpad on day four, whereas those of Tc-specific cells displayed DTH reactions similar to mice that were only immunized with OVA.

After the last measurement of footpad thickness (72 h), the recipients were bled and the levels of Ab in their plasma were analysed by ELISA (IgG1 and IgG2a) or PCA (IgE). All the isotypes were substantially increased in mice that received OVA-specific cells as opposed to the low levels obtained in mice that were not submitted to cell transfer. This effect was not observed in mice injected with Tc-specific cells (Fig. 2).

Profile of cytokines produced by OVA-specific lymphoblasts
To determine the profile of cytokines produced by OVA-specific lymphoblasts, cells isolated by discontinuous Percoll gradient were restimulated in vitro with OVA, Con A or anti-CD3 mAb.

As shown in Table 1, OVA-stimulated cells displayed a Th2 profile, secreting only IL-4 and IL-10. The amount of IL-4 was further increased when the cells were stimulated with Con A or anti-CD3. IL-2 and IFN-γ were only produced under these two latter conditions.

Neutralization of cytokines
We next enriched by negative selection the frequency of OVA-specific T-cell blasts among the cells obtained from the Percoll gradient and transferred i.v. 5.5 × 10^7 cells (72% T-cell blasts) to each mouse of a group of recipients. Mice of another group were injected with 2 × 10^7 cells without further enrichment, containing 23% T-cell blasts. After 12 h, these groups were immunized with OVA in CFA and challenged with aggregated OVA in the footpad on day four. As shown in Figure 3, both groups that received the cell transfer had their DTH reactions completely suppressed in comparison with the group only immunized with OVA, suggesting that OVA-reactive cells responsible for the suppression of DTH are most likely T cells.

Effect of neutralization of IL-4 and/or IL-10 in lymphoblast-transferred mice
Since the OVA-reactive cells secreted IL-4 and IL-10, such cytokines could be suppressing the development of cell-mediated immunity in the recipients of these cells. To address this point, recipient mice were treated with anti-IL-4 mAb 11B11, anti-IL-10 mAb 2A5 or both antibodies. The control groups were injected with normal rat IgG. After 5 h, all the groups were injected i.v. with 2 × 10^7 blast cells and, 12 h later, they were immunized with OVA in CFA. Another group of untreated mice was solely immunized in a similar way.

When the DTH reactions were measured in the footpad after challenge with aggregated OVA on day four (Fig. 4), we observed that neutralization of IL-10 alone did not modify the suppressive effect of OVA-specific blast cells on DTH reactions observed 24, 48 or 72 h after challenge (Fig. 4a). However, the animals that were treated with anti-IL-4 mAb or anti-IL-4 +
anti-IL-10 mAb developed normal DTH responses at 24 h, and the reactions remained elevated until 72 h. Thus, neutralization of IL-4 completely abrogated the suppressive effect of Th2 specific blasts on OVA-specific DTH. The groups that were injected with the control antibody (rat IgG) showed the typical suppression of DTH by cell transfer when compared with the group that was only immunized (Fig. 4a,b).

In vivo neutralization of IL-4 or IL-4 + IL-10 also raised the levels of IL-2, IFN-γ or IL-12 produced by lymph node cells from lymphoblast-transferred mice, after in vitro restimulation with OVA (Table 2).

**Discussion**

The outcome of DTH and antibody responses are inversely correlated. In a previous report we have shown that cell-mediated immunity that develops initially in a non-polarized response to a protein antigen is morphologically modified and gradually abrogated by subsequent development of an antibody response. Among the reported results, we observed that transfer of T-enriched spleen cells, but not sera, from high antibody producers before immunization, abolished the induction of DTH reactions in recipient mice.

In this study, we demonstrate that this effect is mediated by antigen-specific activated T cells through the release of IL-4. Although lymphoblasts expanded by an unrelated antigen, Tc-Ag, did not have any effect on our experimental protocol, the transfer of the same kind of blast cells that produced high levels of IL-4 and IL-10 to C57BL/6 resistant mice just before infection with *T. cruzi* exacerbated the disease, increasing tissue and blood parasite loads and leading to an early death. These results indicate that OVA- and Tc-Ag-specific blast cells must undergo reactivation in vivo by their respective antigens in order to secrete the Th2 type cytokines that counterbalance the signals that drive the response of naive recipient/host T cells in the immunization protocol with OVA + CFA and in *T. cruzi* infection towards a Th1 pattern.

After negative selection, protocols that enriched the frequency of OVA-reactive T-cell blasts from 23 to 72% among the large-size/low-density cells recovered from the Percoll gradient, we demonstrated that transfer of 3.6-fold less cells had a suppressive effect of the same magnitude and that four million activated T cells were sufficient to prevent the induction of DTH. In addition, other large cells, mostly B cells, did not interfere with the suppressive mechanism mediated by T cells, since they represented 54% of the cells before and only 1% after enrichment.

Although the OVA-reactive T-cell blasts secreted IL-4 and IL-10, the latter did not seem to have a major role in the suppressive mechanism because its neutralization did not restore the DTH reactions. This result was not due to lack of effect of the anti-IL-10 mAh, since the same protocol has been used before to neutralize IL-10 in vivo and proved to be effective. Thus, in mice immunized with OVA and *Ascaris suum* extract, we showed that treatment with anti-IL-10 and anti-IL-4 mAb restored the Th1 parameters of the OVA-specific response that were otherwise deeply suppressed, although this did not happen when the mAb were applied singularly. In contrast to IL-10, neutralization of IL-4 alone abolished the dampening effect of OVA-reactive blast cells.
Table 1  Cytokine profile of lymphoblasts from hyperimmune donors

<table>
<thead>
<tr>
<th>Lymphoblasts*</th>
<th>IL-2* (ng/mL ± SD)</th>
<th>IFN-γ (ng/mL ± SD)</th>
<th>IL-10 (U/mL ± SD)</th>
<th>IL-4 (pg/mL ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OVA 250 **</td>
<td>24 h</td>
<td>&lt; 0.4</td>
<td>17.3 ± 2.4</td>
<td>78.4 ± 4.6</td>
</tr>
<tr>
<td>OVA 500</td>
<td></td>
<td>&lt; 0.8</td>
<td>16.7 ± 1.4</td>
<td>80.6 ± 7.2</td>
</tr>
<tr>
<td>OVA 750</td>
<td></td>
<td>&lt; 0.8</td>
<td>16.5 ± 3.5</td>
<td>87.2 ± 7.6</td>
</tr>
<tr>
<td>Con A 5</td>
<td></td>
<td>&lt; 0.4</td>
<td>14.6 ± 1.3</td>
<td>130.2 ± 9.2</td>
</tr>
<tr>
<td>Con A 10</td>
<td></td>
<td>7.7 ± 0.6</td>
<td>15.4 ± 0.1</td>
<td>490.0 ± 15.0</td>
</tr>
<tr>
<td>α-CD3</td>
<td></td>
<td>3.7 ± 1.9</td>
<td>13.5 ± 2.5</td>
<td>1300.0 ± 54.3</td>
</tr>
</tbody>
</table>

*Lymphoblasts from ovalbumin (OVA)-hyperimmune mice cultured for six days with OVA plus IL-2 and purified in Percoll discontinuous gradient.

**Cultures of 5 × 10⁶ cells were restimulated in vitro for 24 or 72 h with OVA (250, 500 or 750 µg/mL), Con A (5 or 10 µg/mL) or anti-CD3 mAb and the supernatants collected. A feeder of 1 × 10⁶ irradiated (3000 rads) spleen cells was used when the cells were incubated with OVA or Con A. Microplate wells were coated with anti-CD3 mAb overnight before cell incubation.

The cytokines were assayed by two-site sandwich ELISA and the results represent the mean of duplicate cultures ± SD. Cytokine levels in unstimulated cell supernatants were: IL-2 < 0.4 ng/mL; IFN-γ < 0.8 ng/mL; IL-10 < 3.1 U/mL; IL-4 < 31.3 pg/mL.

Figure 3  Delayed-type hypersensitivity in recipients of 2 × 10⁶ OVA-specific (OVA-BC) or 5.5 × 10⁶ OVA-specific, T-enriched (OVA-T BC) blast cells from hyperimmune donors, immunized with OVA in CFA after 12 h. Untransferred mice were immunized in the same way (OVA). All the groups, including non-immunized mice, were challenged with aggregated OVA in the footpad on day four. The results represent the arithmetic mean of net increase in footpad thickness of five to six mice ± SEM. *, P < 0.05 compared with OVA-group. ■, non-immunized; (□), OVA; (●), OVA + OVA-BC; (◆), OVA + OVA-T BC.

Figure 3 Delayed-type hypersensitivity in recipients of 2 × 10⁶ OVA-specific (OVA-BC) or 5.5 × 10⁶ OVA-specific, T-enriched (OVA-T BC) blast cells from hyperimmune donors, immunized with OVA in CFA after 12 h. Untransferred mice were immunized in the same way (OVA). All the groups, including non-immunized mice, were challenged with aggregated OVA in the footpad on day four. The results represent the arithmetic mean of net increase in footpad thickness of five to six mice ± SEM. *, P < 0.05 compared with OVA-group. ■, non-immunized; (□), OVA; (●), OVA + OVA-BC; (◆), OVA + OVA-T BC.

Neutralization of IL-4 also increased the amounts of IL-2, IFN-γ and IL-12 produced by lymph node cells of adoptively transferred recipients, indicating a shift back in the direction of a Th1 phenotype. In these experiments it was also observed that, in spite of their IL-4/IL-10-secretory profile, transfer of blast cells increased the levels of IL-2 and IFN-γ in recipients of such cells (treated with normal rat IgG) compared with untransferred recipients. Yet this lymphoblast population contained cells capable of producing these cytokines upon mitogenic or anti-CD3 stimulation that may have been expanded following immunization of the recipients with OVA + CFA.

As regards the increase of OVA-specific IgG1, IgG2a and IgE levels in recipients of OVA-specific lymphoblasts (23% T cells and 54% B cells) on day eight of immunization, our interpretation is that the transferred B-cell blasts are making these antibodies upon restimulation in vivo. This was further supported by the fact that when recipients were injected with T-enriched blast cells containing 72% of T cells and only 1% of B cells their antibody levels were low and similar to those of untransferred mice (data not shown). Inasmuch as DTH reactions were equally suppressed under both cell transfer
IL-4 suppresses induction of early DTH

**Table 2**  Cytokines secreted by lymph node cells from lymphoblast-transferred, OVA-immunized mice treated with anti-IL-4/anti-IL-10 mAb

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>IL-2b (ng/mL ± SD)</th>
<th>IFN-γb (ng/mL ± SD)</th>
<th>IL-12b (ng/mL ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>&lt; 0.4</td>
<td>5.9 ± 0.4</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Rat IgG (6 mg)</td>
<td>1.0 ± 0.0</td>
<td>9.8 ± 0.1</td>
<td>0.5 ± 0.0</td>
</tr>
<tr>
<td>11B11</td>
<td>2.5 ± 0.0</td>
<td>17.9 ± 0.2</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>Rat IgG (8 mg)</td>
<td>1.6 ± 0.0</td>
<td>6.8 ± 0.3</td>
<td>&lt; 0.3</td>
</tr>
<tr>
<td>11B11 + 2A5</td>
<td>2.5 ± 0.0</td>
<td>11.3 ± 0.9</td>
<td>0.6 ± 0.0</td>
</tr>
</tbody>
</table>

aMice were treated with anti-IL-4 (11B11), anti-IL-4 plus anti-IL-10 (11B11 + 2A5) mAb or rat IgG before cell transfer of 2 × 107 OVA-specific blasts and immunization with OVA in CFA. Untreated refers to mice that were only immunized with OVA in CFA.

bThe inguinal and periaortic lymph node cells were collected three days after footpad challenge and incubated at 10 × 10^6 or 6 × 10^6 cells for 24 or 72 h, respectively, with OVA (750 µg/mL). Cytokines were measured by two-site sandwich ELISA in 24 h-(IL-2) and 72 h-supernatants (IFN-γ and IL-12). The results represent the mean of duplicate cultures ± SD. Cytokine levels in unstimulated cell supernatants were: IL-2 < 0.4 ng/mL; IFN-γ < 0.8 ng/mL; IL-12 < 0.3 ng/mL.

glomerulonephritis and adjuvant arthritis. In contrast, IL-4-induced immune deviation may prevent the induction of protective cell-mediated immunity in some vaccination conditions. For example, peripheral blood mononuclear cells from adult recipients of live measles virus vaccine showed spontaneous, PHA-stimulated production of high levels of IL-4, suggesting the preferential activation of Th2 cells. This was associated with a transient depression of DTH skin test responses and mitogen-induced lymphoproliferation in vaccines. In addition, vaccination of mice with a mannoprotein fraction of *Candida albicans*, which contained major immunogenic constituents, resulted in a large number of IL-4-producing CD4+ splenocytes and conferred only a low degree of protection, in contrast with *Candida* whole-cell-vaccinated mice that did not develop a Th2 response and became highly protected.

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**References**
