

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,¹ different parasite antigens^{2,3} or parasite antigen and unrelated antigen.^{4–6} In others, the Th2 cells were induced by IL-4 therapy.^{7,8} 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.⁹ 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.¹⁰ 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 .⁹

According to Rizzo *et al.*¹¹ 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.^{12–14} In addition, IL-10 inhibited vascular leakage and swelling during a DTH reaction induced by injection of Th1 clones into mouse footpads.¹⁵ 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.

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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 (Aldrex; Fontoura-Wyeth, 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-MK₂ cells (American Type Culture Collection, ATCC, CCL7) and submitted to 10 cycles of freezing and thawing, as previously described.¹⁶ 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.¹⁷ 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 $\pm$ SEM of each group. A two-way analysis of variance followed by multiple comparisons using the Tukey method¹⁸ 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 $\times 10^6$ 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 $\times 10^6$ cells) or 72 h (6 $\times 10^6$ 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.

Titration 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 antimouse monospecific 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 ($\pm$ 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.¹⁸

Titration of IgE antibodies by PCA

IgE antibodies were measured by passive cutaneous anaphylaxis (PCA) as described by Mota and Wong.¹⁹ PCA 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.^{20,21}

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 $\times 10^7$ parasites/animal) and same amount of adjuvant and were boosted on days 14 and 28 with Tc-Ag in PBS (5 $\times 10^6$ 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 $\times 10^7$ 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 flasks 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.^{22,23} 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 $\times 10^7$ 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.⁹ 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 injected 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.

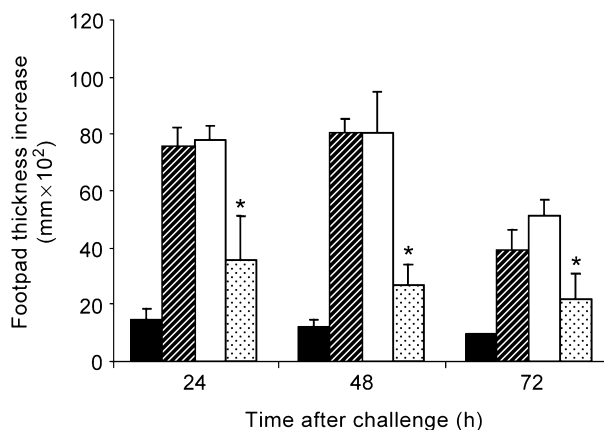


Figure 1 Delayed-type hypersensitivity (DTH) in recipient mice injected i.v. with 1.2×10^7 ovalbumin (OVA)-specific (OVA-BC) or Tc-specific (Tc-BC) lymphoblasts from hyperimmune donors, and immunized with OVA in complete Freund's adjuvant (CFA) after 12 h. Untransferred mice were immunized in the same way (OVA). All the groups, including a group of 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 $\pm$ SEM. ★, $P < 0.05$ compared with OVA-group. (■), non immunized; (▨), OVA; (□), OVA + Tc-BC; (▤), OVA + OVA-BC.

Suppressive effect of enriched T-cell blasts

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. 5.5×10^6 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 +

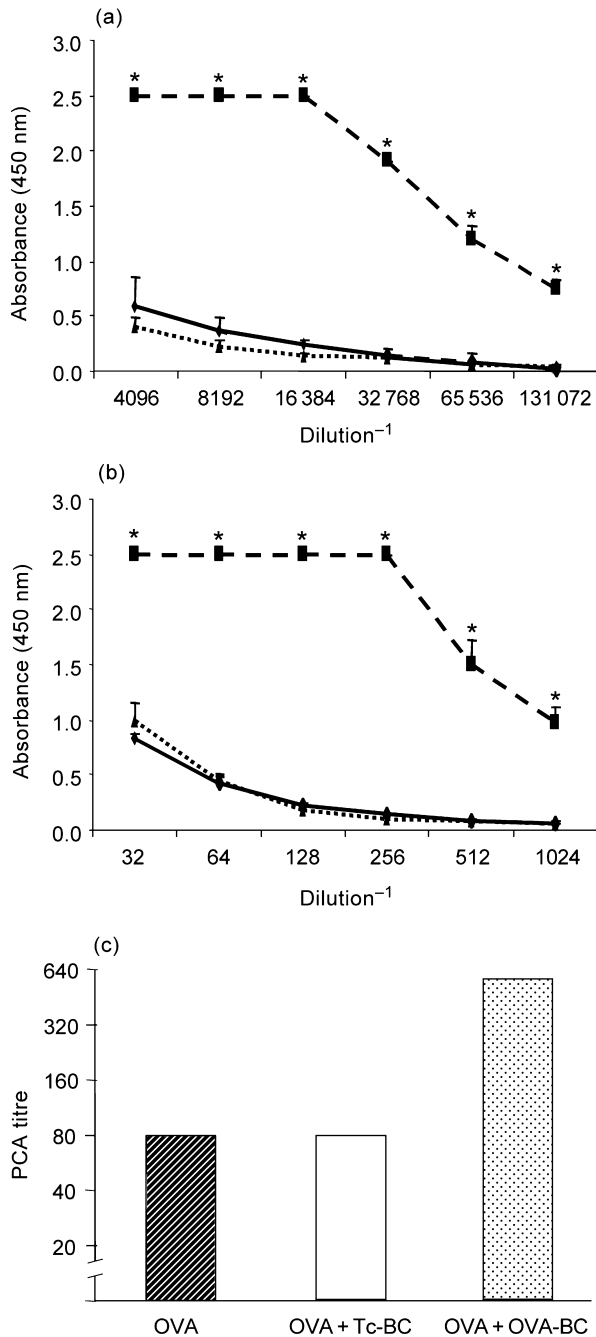


Figure 2 Ovalbumin-specific IgG1 (a), IgG2a (b) and IgE (c) antibodies produced by recipients of 1.2×10^7 OVA-specific (OVA-BC) or Tc-specific (Tc-BC) lymphoblasts, immunized with OVA in CFA after 12 h. These antibodies were also measured in untransferred mice immunized in the same way (OVA). The levels of IgG1 and IgG2a were determined in plasma by sandwich ELISA and the results represent the mean $\pm$ SEM absorbance of five to six mice. IgE was titrated by passive cutaneous anaphylaxis (PCA) and the results represent the reciprocal of the highest dilution of pooled plasma from five to six mice that gave a lesion of >5 mm in diameter. ★, $P < 0.05$ compared with OVA-group. ◆, OVA; ▲, OVA + Tc-BC; ■, OVA + OVA-BC.

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.^{11,24,25} In a previous report⁹ 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.²⁶ 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 mAb, 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,²⁷ 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

Lymphoblasts ^a	IL-2 ^c (ng/mL $\pm$ SD)	IFN- γ (ng/mL $\pm$ SD)	IL-10 (U/mL $\pm$ SD)	IL-4 (pg/mL $\pm$ SD)
	24 h		72 h	
OVA 250 ^b	< 0.4	< 0.8	17.3 $\pm$ 2.4	78.4 $\pm$ 4.6
OVA 500	< 0.4	< 0.8	16.7 $\pm$ 1.4	80.6 $\pm$ 7.2
OVA 750	< 0.4	< 0.8	16.5 $\pm$ 3.5	87.2 $\pm$ 7.6
Con A 5	< 0.4	1.4 $\pm$ 0.1	14.6 $\pm$ 1.3	130.2 $\pm$ 9.2
Con A 10	7.7 $\pm$ 0.6	2.1 $\pm$ 0.1	15.4 $\pm$ 0.1	490.0 $\pm$ 15.0
α -CD3	3.7 $\pm$ 1.9	6.4 $\pm$ 0.2	13.5 $\pm$ 2.5	1300.0 $\pm$ 54.3

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

^bCultures of 5×10^6 cells were restimulated *in vitro* for 24 or 72 h with OVA (250, 500 or 750 μ g/mL), Con A (5 or 10 μ g/mL) or anti-CD3 mAb and the supernatants collected. A feeder of 1×10^7 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.

^cThe cytokines were assayed by two-site sandwich ELISA and the results represent the mean of duplicate cultures $\pm$ 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.

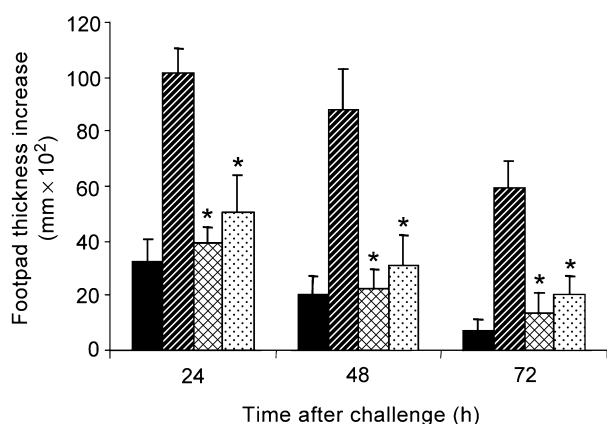


Figure 3 Delayed-type hypersensitivity in recipients of 2×10^7 OVA-specific (OVA-BC) or 5.5×10^6 OVA-specific, T-enriched (OVA-T BC) blast cells from hyperimmune donors, immunized with OVA in CFA after 12 h. Untransferred mice were immunized in the same way (OVA). All the groups, including non-immunized 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 $\pm$ SEM. ★, $P < 0.05$ compared with OVA-group. ■, non-immunized; (▨), OVA; (▩), OVA + OVA-BC; (░), OVA + OVA-T BC.

on DTH development, indicating therefore that this cytokine is the main mediator of such effect. In addition, this result further supports the conclusion that IL-4-producing T cells are the cells that suppress DTH. Concomitant neutralization of IL-4 and IL-10 did not make any further difference that could suggest a synergistic action. These results differ from those of some previous studies. Inhibition of Th1 development induced by heat-killed *Listeria monocytogenes* can occur due to endogenous IL-10 as well as IL-4 production during the priming phase of CD4⁺ T cells.²⁸ IL-4 and IL-10 also synergize, inhibiting the effector function of Th1 cells in a tuberculin-type DTH response to *Leishmania major*.¹² Irritant and contact hypersensitivity responses, on the other hand, are naturally regulated by IL-10.^{29,30} However, Powrie *et al.*³¹ reported that administration of anti-IL-4, but not of anti-

IL-10, to *L. major*-infected severe combined immunodeficient (SCID) mice transferred with a mixture of CD45RB^{high} and CD45RB^{low} cells led to the development of protective Th1 responses, indicating that the regulatory role of adoptively transferred Th2 cells is mediated by an IL-4-dependent mechanism. Our results corroborate these latter findings, since they show that expansion of CD4⁺ T cells following encounter with antigen in the presence of IL-4, produced by the adoptively transferred Th2 type cells, changed the profile of the immune response, thus leading to suppression of DTH. Neutralization of IL-4 alone not only restored the DTH reaction, but also maintained it in a plateau at least until 72 h after challenge. This result suggests that as the effector phase of this reaction reaches the maximum it is downregulated by this cytokine in order to limit inflammation and tissue damage. Corroborating this interpretation are our findings that OVA-specific DTH reactions in IL-4 knockout mice are also of a higher magnitude and also maintained for a longer period of time than in wild-type mice immunized with OVA + CFA (Jacysyn and Macedo, manuscript in preparation).

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

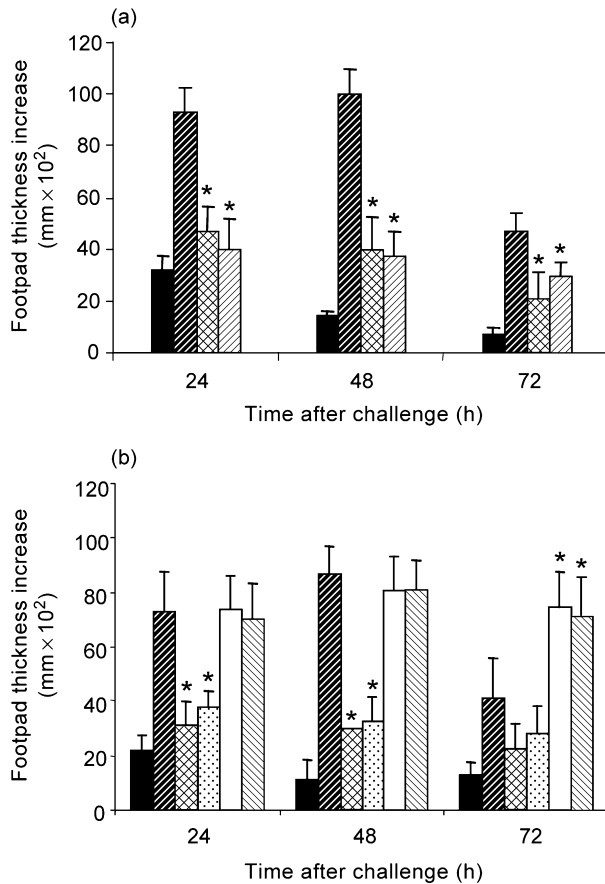


Figure 4 Effect of neutralization of (a) IL-10 (2A5) and (b) IL-4 (11B11) or IL-4 + IL-10 (11B11 + 2A5) on DTH of recipient mice. Cytokine-specific antibodies or normal rat IgG were injected 5 h before cell transfer. Recipients were injected with 2×10^7 OVA-specific blast cells from hyperimmune donors and immunized with OVA in CFA after 12 h. Untreated and untransferred mice were immunized in the same way (Untreated). All the groups, including a group of 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 $\pm$ SEM. ★, $P < 0.05$ compared with Untreated-group. (a) (■), non-immunized; (▨), untreated; (▩), rat IgG (2 mg); (▧), 2A5. (b) (■), non-immunized; (▨), untreated; (▩), rat IgG (6 mg); (▧), rat IgG (8 mg); (□), 11B11; (▩), 11B11 + 2A5.

conditions, these results reinforce once more that T cells, but not antibodies, were responsible for the inhibition of DTH induction. We cannot exclude, however, that the transferred Ag-reactive T cells could help recipient B cells to make more antibody later on.

In conclusion, the results presented here indicate that activation of Ag-specific Th2 cells early in the course of the immune response to a single defined antigen increases the levels of IL-4, that consequently favours the development of humoral immunity to the detriment of Th1-mediated immunity against the same antigen. It has been shown that this endogenous IL-4 may attenuate Th1-mediated injurious inflammatory responses such as detected in crescentic

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

Treatment ^a	IL-2 ^b (ng/mL $\pm$ SD)	IFN- γ ^b (ng/mL $\pm$ SD)	IL-12 ^b (ng/mL $\pm$ SD)
Untreated	< 0.4	5.9 $\pm$ 0.4	0.9 $\pm$ 0.1
Rat IgG (6 mg)	1.0 $\pm$ 0.0	9.8 $\pm$ 0.1	0.5 $\pm$ 0.0
11B11	2.5 $\pm$ 0.0	17.9 $\pm$ 0.2	0.9 $\pm$ 0.1
Rat IgG (8 mg)	1.6 $\pm$ 0.0	6.8 $\pm$ 0.3	< 0.3
11B11 + 2A5	2.5 $\pm$ 0.0	11.3 $\pm$ 0.9	0.6 $\pm$ 0.0

^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 2×10^7 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 μ g/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 $\pm$ 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.^{32,33} 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 vaccinees.³⁴ 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.³⁵

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References

- Pearce EJ, Caspar P, Grzych JM, Lewis FA, Sher A. Down-regulation of the Th1 cytokine production accompanies induction of Th2 response by a parasitic helminth, *Schistosoma mansoni*. *J. Exp. Med.* 1991; **173**: 159–66.
- Curry AJ, Else KJ, Jones F, Bancroft A, Grecis RK, Dunne DW. Evidence that cytokine-mediated immune interaction induced by *Schistosoma mansoni* alter disease outcome in mice concurrently infected by *Trichuris muris*. *J. Exp. Med.* 1995; **181**: 769–74.
- Yan Y, Inuo G, Akao N, Tsukidate S, Fujita K. Down-regulation of murine susceptibility to cerebral malaria by inoculation with third-stage larvae of the filarial nematode *Brugia pahangi*. *Parasitology* 1997; **114**: 333–8.

- 4 Kullberg MC, Pearce EJ, Hieny SE, Sher A, Berzofsky JA. Infection with *Schistosoma mansoni* alters Th1/Th2 cytokine response to a non-parasite antigen. *J. Immunol.* 1992; **148**: 3264–70.
- 5 Pearlman E, Kazura J, Hazlett Jr FE, Boom WH. Modulation of murine cytokine responses to mycobacterial antigens by helminth-induced T helper 2 cell response. *J. Immunol.* 1993; **151**: 4857–64.
- 6 Actor JK, Marshall MA, Eltoun IA, Buller RML, Berzofsky JA, Sher A. Increased susceptibility of mice infected with *Schistosoma mansoni* to recombinant vaccinia virus: association of viral persistence with egg granuloma formation. *Eur. J. Immunol.* 1994; **24**: 3050–6.
- 7 Racke MK, Bonomo A, Scott DE *et al.* Cytokine-induced immune deviation as therapy for inflammatory autoimmune disease. *J. Exp. Med.* 1994; **180**: 1961–6.
- 8 Biedermann T, Maillhammer R, Mai A *et al.* Reversal of established delayed type hypersensitivity reactions following therapy with IL-4 or antigen-specific Th2 cells. *Eur. J. Immunol.* 2001; **31**: 1582–91.
- 9 Jacysyn JF, Abrahamsohn IA, Macedo MS. Modulation of delayed-type hypersensitivity during the time course of immune response to a protein antigen. *Immunology* 2001; **102**: 373–9.
- 10 Alexander J, Curtis J. Development of delayed hypersensitivity responses in *Mycobacterium lepraemurium* infections in resistant and susceptible strains of mice. *Immunology* 1979; **36**: 563–7.
- 11 Rizzo LV, Dekruyff RH, Umetsu DT, Caspi RR. Regulation of the interaction between Th1 and Th2 T cell clones to provide help for antibody production in vivo. *Eur. J. Immunol.* 1995; **25**: 708–16.
- 12 Powrie F, Menon S, Coffman RL. Interleukin-4 and interleukin-10 synergize to inhibit cell-mediated immunity *in vivo*. *Eur. J. Immunol.* 1993; **23**: 2223–9.
- 13 Mu H-H, Sewell WA. Regulation of DTH and IgE responses by IL-4 and IFN- γ in immunised mice given pertussis toxin. *Immunology* 1994; **83**: 639–45.
- 14 Flores-Villanueva PO, Zheng XX, Strom TB, Staderker MJ. Recombinant IL-10 and IL-10/Fc treatment down-regulate egg antigen-specific delayed hypersensitivity reactions and egg granuloma formation in schistosomiasis. *J. Immunol.* 1996; **156**: 3315–20.
- 15 Li L, Elliott JF, Mosmann TR. IL-10 inhibits cytokine production, vascular leakage and swelling during T helper 1 cell-induced delayed-type hypersensitivity. *J. Immunol.* 1994; **153**: 3967–78.
- 16 Curotto de Lafaille MA, Barbosa de Oliveira LC, Lima GC, Abrahamsohn IA. *Trypanosoma cruzi*: maintenance of parasite-specific T cell responses in lymph nodes during the acute phase of infection. *Exp. Parasitol.* 1990; **70**: 164–74.
- 17 Ferreira AP, Faquim ES, Abrahamsohn IA, Macedo MS. Immunization with *Ascaris suum* extract impairs T cell functions in mice. *Cell. Immunol.* 1995; **162**: 202–10.
- 18 Zar JH. *Biostatistical Analysis*. New York: Prentice Hall 1984.
- 19 Mota I, Wong D. Homologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci.* 1969; **8**: 813–20.
- 20 Fox DA, Chiorazzi N, Katz DH. Hapten-specific IgE antibody response in mice. V. Differential resistance of IgE and IgG B lymphocytes to X-irradiation. *J. Immunol.* 1976; **117**: 1622–8.
- 21 Ishizaka K, Adachi T. Generation of specific helper cells and suppressor cells *in vitro* for the IgE and IgG antibody response. *J. Immunol.* 1976; **117**: 40–7.
- 22 Kurnick JT, Grönvik KO, Kimura AK. Long term growth *in vitro* of human T-cell blasts with maintenance of specificity and function. *J. Immunol.* 1979; **122**: 1255–60.
- 23 Louis JA, Moedder E, MacDonald HR, Engers HD. Recognition of protozoan parasites by murine T lymphocytes. II. Role of the H-2 gene complex in interactions between antigen-presenting macrophages and Leishmania-immune T lymphocytes. *J. Immunol.* 1981; **126**: 1661–6.
- 24 Parish CR, Liew FY. Immune response to chemically modified flagellin. III. Enhanced cell-mediated immunity during high and low zone tolerance to flagellin. *J. Exp. Med.* 1972; **135**: 298–311.
- 25 Silver J, Benacerraf B. Dissociation of T cell helper function and delayed hypersensitivity. *J. Immunol.* 1974; **113**: 1872–5.
- 26 Barbosa de Oliveira LC, Curotto de Lafaille MA, Lima GMCA, Abrahamsohn IA. Antigen-specific IL-4- and IL-10-secreting CD4⁺ lymphocytes increase *in vivo* susceptibility to *Trypanosoma cruzi* infection. *Cell. Immunol.* 1996; **170**: 41–53.
- 27 Macedo MS, Faquim-Mauro E, Ferreira AP, Abrahamsohn IA. Immunomodulation induced by *Ascaris suum* extract in mice. effect of anti-interleukin-4 and anti-interleukin-10 antibodies. *Scan. J. Immunol.* 1998; **47**: 10–18.
- 28 Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of T_H1 CD4⁺ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 1993; **260**: 547–9.
- 29 Ferguson TA, Dube P, Griffith TS. Regulation of contact hypersensitivity by interleukin 10. *J. Exp. Med.* 1994; **179**: 1597–604.
- 30 Berg DJ, Leach MW, Kuhn R *et al.* Interleukin 10 but not interleukin 4 is a natural suppressant of cutaneous inflammatory responses. *J. Exp. Med.* 1995; **182**: 99–108.
- 31 Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. Regulatory interactions between CD45RB^{high} and CD45RB^{low} CD4⁺ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J. Exp. Med.* 1994; **179**: 589–600.
- 32 Kitching AR, Tipping PG, Mutch DA, Huang XR, Holdsworth SR. Interleukin-4 deficiency enhances Th1 responses and crescentic glomerulonephritis in mice. *Kidney Int.* 1998; **53**: 112–8.
- 33 Yoshino S, Murata Y, Ohsawa M. Successful induction of adjuvant arthritis in mice by treatment with a monoclonal antibody against IL-4. *J. Immunol.* 1998; **161**: 6904–8.
- 34 Ward BJ, Griffin DE. Changes in cytokine production after measles virus vaccination: predominant production of IL-4 suggests induction of a Th2 response. *Clin. Immunol. Immunopathol* 1993; **67**: 171–7.
- 35 Mencacci A, Torosantucci A, Spaccapelo R, Romani L, Bistoni F, Cassone A. A mannoprotein constituent of *Candida albicans* that elicits different levels of delayed-type hypersensitivity, cytokine production, and anticandidal protection in mice. *Infect. Immun* 1994; **62**: 5353–60.