

# Helper Activity for Immunoglobulin Synthesis of T Helper Type 1 (Th1) and Th2 Human T Cell Clones: The Help of Th1 Clones Is Limited by their Cytolytic Capacity

By Gianfranco F. Del Prete, Marco De Carli, Mario Ricci, and Sergio Romagnani

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From the Division of Clinical Immunology and Allergology, Istituto di Clinica Medica 3, University of Florence, I-50134 Florence, Italy

## Summary

A large number of CD4<sup>+</sup> human T helper type 1 (Th1) clones specific for purified protein derivative and of Th2 clones specific for the excretory/secretory antigen of *Toxocara canis*, derived from the same individuals, were analyzed for both cytotoxic capacity and helper function for immunoglobulin (Ig) synthesis. The great majority of Th1, but only a minority of Th2 clones exhibited cytolytic activity. All Th2 (noncytolytic) clones induced IgM, IgG, IgA, and IgE synthesis by autologous B cells in the presence of the specific antigen, and the degree of response was proportional to the number of Th2 cells added to B cells. Under the same experimental conditions, Th1 (cytolytic) clones provided helper function for IgM, IgG, and IgA, but not IgE, synthesis with a peak response at 1:1 T/B cell ratio. At higher T/B cell ratios, a strong decrease of Ig synthesis was observed. All Th1 clones lysed Epstein-Barr virus transformed autologous B cells pulsed with the specific antigen. The decrease of Ig production at high T/B cell ratios correlated with the lytic activity of Th1 clones against autologous antigen-presenting B cell targets. These data suggest that Th1 differ from Th2 human T cell clones not only for their profile of cytokine secretion, but also for cytolytic potential and mode of help for B cell Ig synthesis.

CD4<sup>+</sup> murine Th cells have been categorized into at least two distinct subsets based on their profiles of cytokine secretion (1, 2). One type of Th (Th1) produces IL-2 and IFN- $\gamma$ , whereas the other type (Th2) secretes IL-4 and IL-5, but not IL-2 and IFN- $\gamma$  (1, 2). In addition to produce different sets of cytokines, murine Th1 and Th2 cells display different functional activities. Th1 cells mediate cytotoxicity (in vitro) (3), augment the production of antigen-specific responses by hapten-primed B cells (4), and stimulate bystander antibody production (5). Th2 cells appear to be more effective than Th1 cells in providing help for primary antigen-specific Ig secretion (6). Moreover, IL-4 produced by Th2 cells selectively induces B cell switching to the IgE isotype (7).

We have recently provided evidence for the existence of Th1 and Th2 subpopulations in human CD4<sup>+</sup> lymphocytes (8). This has been done by establishing a large series of T cell clones specific for purified protein derivative (PPD)<sup>1</sup> or *Toxocara canis* excretory/secretory (TES) antigens from PBL of two healthy individuals. Virtually all PPD-specific T cell clones expressed RNA for, and secreted into the supernatants,

IL-2 and IFN- $\gamma$ , but not IL-4 or IL-5. In contrast, the great majority of TES-specific clones expressed RNA for, and secreted, IL-4 and IL-5, but not IL-2 and IFN- $\gamma$  (8). In the present study, the functional properties of these two sets of clones have been examined. Data showed that all Th1 and Th2 clones induced IgM, IgG, and IgA synthesis, whereas only Th2 clones induced IgE synthesis in autologous B cells in the presence of the specific antigen. However, at high T/B cell ratios, the helper function for Ig synthesis of Th1, but not of Th2, clones strongly declined. Stimulation with the specific antigen under MHC-restricted conditions enabled the great majority of Th1, but only a few Th2, clones to express cytolytic activity against autologous APC. The results also demonstrated that the decline in the helper activity for Ig synthesis of Th1 correlated with their ability to kill antigen-presenting autologous B cells.

## Materials and Methods

**Reagents.** PHA was purchased by Gibco Laboratories (Grand Island, NY). Human rIL-2 was kindly provided by Eurocetus (Milan, Italy). PPD was kindly provided by Istituto Sieroterapico e Vac-

<sup>1</sup> Abbreviations used in this paper: PPD, purified protein derivative; SN, supernatant; TES, *Toxocara canis* excretory/secretory.

cinogeno Sclavo (Siena, Italy). The excretory/secretory antigen(s) of *Toxocara canis* (TES) was prepared as described by De Savigny (9).

**Generation of Antigen-specific T Cell Clones.** Antigen-specific T cell clones specific for PPD or TES were generated from PBL of two individuals without history of overt tuberculosis or toxocarasis, but showing both delayed-type hypersensitivity skin reaction to PPD and in vitro proliferative response of their PBL to TES antigen, as previously described (8).

**Preparation of B Cells and Immobilization of B Cells with EBV.** B cell-enriched suspensions were prepared as described in detail elsewhere (10). PBMC were isolated by flotation over Ficoll-Hypaque, and phagocytic cells were removed by adherence to plastic flasks. T lymphocytes were removed by a double step of rosetting with SRBC followed by C'-dependent cytotoxicity with anti-CD3 mAb plus fresh rabbit serum. Peripheral blood B cell-enriched suspensions usually consisted of 55-75% B cells, 3-10% monocytes, and <1% T cells. They will be referred to as B cells. To obtain EBV-transformed lymphoblastoid B cell lines (EBV-B cells), B cells were incubated for 48 h with supernatant (SN) of the EBV-producing marmoset cell line B95.8 and subsequently expanded in complete medium supplemented with 15% FCS.

**Assays for Cytolytic Activity.** Cytolytic activity of clones was assessed as reported elsewhere (11). Briefly, T blasts were washed thrice, resuspended, counted, and divided into two aliquots. One aliquot was tested for cytolytic activity against murine <sup>51</sup>Cr-labeled P815 mastocytoma cells in the presence of PHA (1% vol/vol) (lectin-dependent cytolytic assay) with E/T ratio of 4:1. After 4 h at 37°C, 0.1 ml SN was removed for measurement of <sup>51</sup>Cr release. Maximum release (MR) was obtained by treating target cells with 0.1 ml 1 M HCl. Spontaneous release (SR) was determined in control microcultures without effector cells. Specific lysis was calculated according to the formula: percent specific lysis = 100 × (Experimental release - SR)/(MR - SR). Cultures in which <sup>51</sup>Cr release exceeded the mean SR by >5 SD were considered positive for cytolytic activity. To assess the antigen-induced cytolytic activity of clones against autologous APC, EBV-B cells were incubated overnight with PPD or TES (5 µg/ml), pelleted, resuspended, and labeled with <sup>51</sup>Cr (1 h at 37°C). After three washes, 10<sup>4</sup>/0.1 ml target cells were distributed in triplicate wells of 96-well V-bottomed plates. The second aliquot of washed T blasts from each clone was then added in 0.1 ml at E/T ratios from 1:1 to 16:1. After centrifugation, microplates were incubated for 4 h at 37°C, and 0.1 ml SN was removed for measurement of <sup>51</sup>Cr release. The percent specific lysis was calculated using the formula reported above.

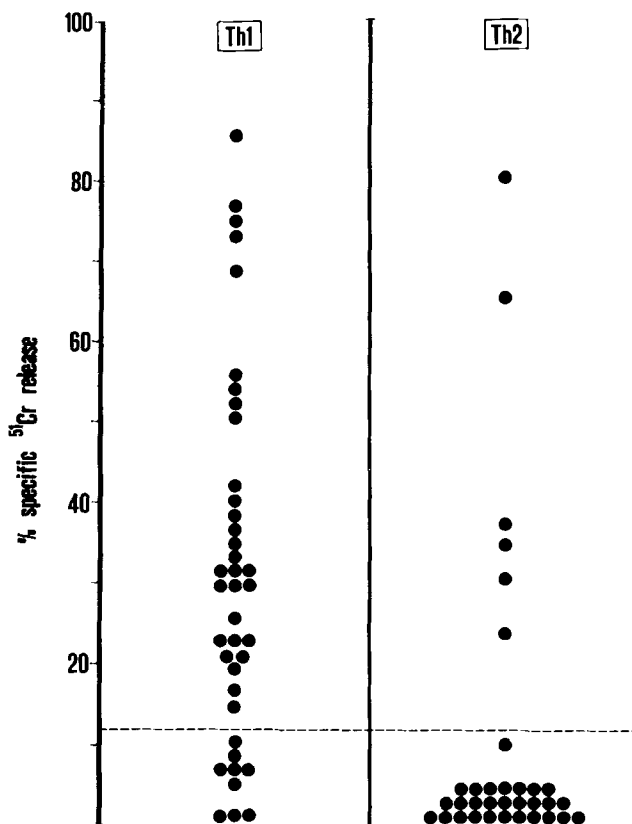
**Assay for Helper Function of T Cell Clones.** The cell culture system used for the induction of Ig synthesis was performed in duplicate 12 × 75-mm plastic tubes (Falcon Labware, Oxnard, CA) by using complete medium supplemented with 10% FCS. Each tube contained 5 × 10<sup>4</sup> B cells and different concentrations of clonal T blasts (from 1.25 × 10<sup>4</sup> to 8 × 10<sup>5</sup>) in 1 ml. After 10 d, culture SN were collected and assayed for their Ig content. The immunoradiometric assay used for detecting IgM, IgG, and IgA has been described previously in detail (12). The procedure for the measurement of IgE has also been described in detail elsewhere (13).

## Results and Discussion

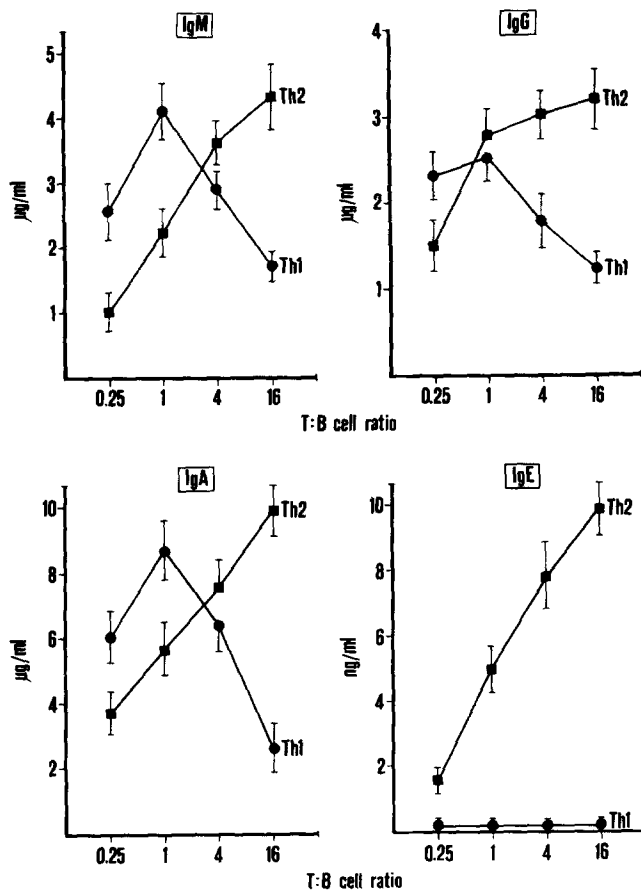
We have recently generated a large panel of PPD-specific and TES-specific CD4<sup>+</sup> T cell clones from PBL of two healthy individuals (8). PPD-specific clones secreted IL-2 and IFN-γ but not IL-4 or IL-5 after stimulation with the specific antigen, and were categorized as Th1. Under the same ex-

perimental conditions, TES-specific clones produced IL-4 and IL-5 but not IL-2 or IFN-γ, and were categorized as Th2 (8). In this study, we have assessed the cytolytic potential of 39 PPD-specific (Th1) and 34 TES-specific (Th2) clones in a lectin (PHA)-dependent 4-h <sup>51</sup>Cr-release assay with P815 murine mastocytoma cells as target. At an E/T ratio of 4:1, the great majority (77%) of Th1, but only a few (18%) Th2, clones showed cytolytic activity (Fig. 1). This finding confirms the results of previous observations in both mouse (3) and humans (14).

10 cytolytic Th1 and 10 noncytolytic Th2 clones were then selected and tested at different concentrations (from 1.25 × 10<sup>4</sup> to 0.8 × 10<sup>6</sup>/ml) for their ability to induce IgM, IgG, IgA, and IgE synthesis by autologous B cells (5 × 10<sup>4</sup>/ml) in the absence or in the presence of PPD (2 µg/ml) or TES (2 µg/ml), respectively. In the absence of their specific antigen, both Th1 and Th2 clones exerted poor or no helper function for Ig synthesis (data not shown). As shown in Fig. 2, antigen stimulation enabled all Th2 clones to provide helper function for IgM, IgG, IgA, and IgE synthesis, and the degree of response was proportional to the number of Th2 cells added to B cells. As expected, at any T/B cell ratio, Th1 clones did not induce IgE synthesis. This finding is consistent with



**Figure 1.** Cytolytic activity of individual CD4<sup>+</sup> Th1 (PPD-specific) and Th2 (TES-specific) clones derived from PBL of two healthy donors. Cytolytic activity of each clone was tested against <sup>51</sup>Cr-labeled P815 target cells at an E/T ratio of 4:1 in the presence of PHA. Specific lysis was calculated as described in Materials and Methods. The dotted line represents 5 SD above the mean spontaneous release of target cells.

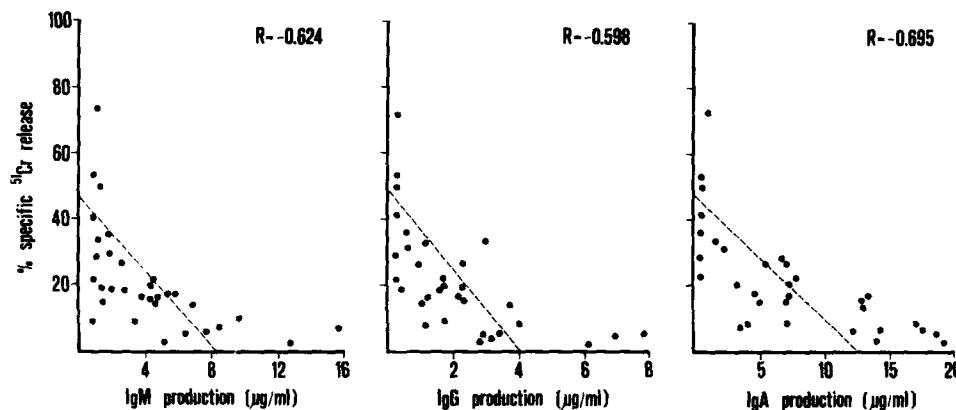


**Figure 2.** In vitro synthesis of IgM, IgG, IgA, and IgE induced in autologous B cells by different concentrations of Th1 (PPD-specific) and Th2 (TES-specific) clones stimulated with the specific antigen. Cloned T cells (from  $1.25 \times 10^4$  to  $8 \times 10^5$ ) were cultured with autologous B cells ( $5 \times 10^4$ ) in 1 ml in the presence of the specific antigen ( $2 \mu\text{g/ml}$ ). After 10 d, culture SN were recovered and assayed for their Ig content, as reported in Materials and Methods. The results represent the mean ( $\pm$  SE) values obtained with 10 Th1 and 10 Th2 clones.

the demonstration that IL-4 is required for the induction of both murine and human IgE antibody synthesis, whereas IFN- $\gamma$  suppresses the IL-4-dependent IgE production (13, 15). Accordingly, the addition in culture of both rIL-4 and anti-

IFN- $\gamma$  antibody enabled even PPD-stimulated Th1 clones to induce IgE synthesis by autologous B cells (data not shown).

The pattern of Th1 help on the antigen-induced production of IgM, IgG, and IgA was unexpected. At low T/B cell ratios, there was substantial production of IgM, IgG, and IgA with a peak response at 1:1 ratio. At higher T/B cell ratios, a strong reduction of Ig synthesis was consistently observed (Fig. 2). Since the decline of helper activity at high T/B cell ratios was a peculiar property of cytolytic clones, we hypothesized that it could be due to the concomitant killing by Th1 clones of antigen-presenting autologous B cells. This question was addressed in two ways. First, in four experiments, the viability of B cells cocultured with PPD-pulsed Th1 or TES-pulsed Th2 clones at a 4:1 T/B ratio was assessed at 4 and 48 h by the Trypan blue dye exclusion technique. As expected, the viability of B cells cocultured with Th1 clones was lower (range, 74–46% after 4 h, and 56–33% after 48 h) than that found in cocultures of B cells with Th2 clones (range 98–95% and 96–93% after 4 and 48 h, respectively). As an additional approach to prove that the decline of helper activity by Th1 clones at high T/B cell ratios resulted from the killing of antigen-presenting B cells, PPD-pulsed autologous EBV-induced lymphoblastoid B cells were used as target cells for PPD-specific Th1 clones in a 4-h  $^{51}\text{Cr}$ -release assay. All six Th1 clones tested, which were cytolytic for P815 cells in the lectin-dependent assay, also lysed autologous EBV-B cells pulsed with the specific antigen (mean percent specific  $^{51}\text{Cr}$  release  $\pm$  SD:  $18.4 \pm 4$  at 1:1 T/B ratio;  $35.3 \pm 9$  at 4:1 ratio; and  $59.7 \pm 13$  at 20:1 ratio), but not the same targets pulsed with irrelevant (TES) or no antigen (<5%). More importantly, a significant inverse correlation ( $p < 0.0005$ ) between the lytic activity of Th1 clones against autologous antigen-pulsed EBV-B cell targets and their ability to help IgM (R:  $-0.624$ ), IgG (R:  $-0.598$ ), and IgA (R:  $-0.695$ ) synthesis by fresh autologous B cells pulsed with the same antigen was found (Fig. 3). The activity of Th1 clones in mediating killing of APC might provide an explanation for the controversy regarding their capacity to provide antigen-specific cognate help to B cells (5, 16, 17). It is indeed possible that different antigen concentrations and cell densities used by different investigators have influenced the efficiency of APC killing by Th1. More importantly, our



**Figure 3.** Ability of PPD-stimulated Th1 clones to induce Ig synthesis vs. their capacity to lyse autologous EBV-B cells pulsed with the specific antigen. Values of IgM, IgG, and IgA synthesis induced by PPD-stimulated T blasts ( $2.5 \times 10^5/\text{ml}$ ) in autologous B cells ( $5 \times 10^4/\text{ml}$ ) were plotted against values of percent specific  $^{51}\text{Cr}$  release by antigen-pulsed EBV-B cells at a T/B ratio of 5:1. The experimental conditions were the same described in the legends of Fig. 2 and Table 1.

data suggest that Th1 cells may be involved not only in the induction but also in the downregulation of specific B cell responses.

The cytotoxic effect exerted by Th1 clones against antigen-presenting B cells might be due to direct cytotoxicity (18, 19) or production of soluble cytokines, such as TNF- $\alpha$ , TNF- $\beta$ , and IFN- $\gamma$  (20). Since a mixture of high amounts of antibodies specific for human IL-2, IFN- $\gamma$ , TNF- $\alpha$ , and TNF- $\beta$  did not affect the antigen-induced cytotoxicity of Th1 clones against autologous EBV-B cells (data not shown), it is likely that the set of Th1-derived cytokines plays a minor role, if any, in the cytotoxic activity of Th1 clones. This possibility was further supported by experiments in which the effect of Th1 SN vs. Th1 cells on the Th2-induced antibody response was evaluated. When PPD-activated Th1 clones were added to cocultures of B cells plus TES-activated Th2 clones, the amounts of secreted Ig were lower than those expected (Table 1). In contrast, the addition to cocultures of B cells and TES-activated Th2 clones of SN from the same PPD-stimulated Th1 clones consistently resulted in an Ig synthesis higher than expected (Table 1), likely due to the activity of IL-2 and IFN- $\gamma$ , present in Th1 SN, in supporting IgM, IgG, and IgA production.

These data suggest that lymphokine-mediated effector activity and cytotoxicity are distinct functions of Th1 cells. However, due to both these functions, Th1 cells represent the most important effector cells in inflammatory reactions associated with vigorous delayed-type hypersensitivity responses and low antibody titers like those occurring in contact dermatitis or in some infections by intracellular bacteria. In contrast, the functional phenotype of Th2 cells (noncytolytic, high IL-4 and IL-5, no IFN- $\gamma$  production) may account for both the persistent production of antibodies (including IgE) and eosinophilia observed in helminthic infections and allergic disorders.

**Table 1.** Effect of Addition of PPD-stimulated Th1 Cells or their SN on Ig Production by B Cells Cocultured with TES-stimulated Th2 Cells

B cells cultured with:	Ig production		
	IgM	IgG	IgA
		$\mu\text{g/ml}$	
Medium	< 0.1	< 0.1	0.2
Th2 cells	1.9 $\pm$ 0.4	1.4 $\pm$ 0.4	2.7 $\pm$ 0.8
Th1 cells	1.0 $\pm$ 0.3	1.1 $\pm$ 0.3	1.6 $\pm$ 0.5
Th1 SN	0.9 $\pm$ 0.3	0.8 $\pm$ 0.3	0.6 $\pm$ 0.5
Th2 cells + Th1 cells (expected)	2.9 $\pm$ 0.6	2.5 $\pm$ 0.7	4.3 $\pm$ 1.3
Th2 cells + Th1 cells (actual)	1.7 $\pm$ 0.3	1.4 $\pm$ 0.3	2.2 $\pm$ 0.3
Th2 cells + Th1 SN (expected)	2.8 $\pm$ 0.7	2.2 $\pm$ 0.6	3.3 $\pm$ 1.0
Th2 cells + Th1 SN (actual)	6.3 $\pm$ 2.4	3.7 $\pm$ 0.7	5.4 $\pm$ 1.4

B cells ( $5 \times 10^4$ ) were cocultured with TES-activated T blasts ( $2 \times 10^5$ ) from autologous Th2 clones, in the presence or in the absence of PPD-activated T blasts ( $2 \times 10^5$ ) from autologous Th1 clones. SN from the same Th1 clones, stimulated for 24 h with PPD, were also tested (50% final concentration) under the same experimental conditions. After 10 d, Ig production was measured as reported in Materials and Methods. The results represent the mean ( $\pm$  SD) values obtained by mixing each of three Th2 clones with four Th1 clones or their SN.

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Address correspondence to Sergio Romagnani, Istituto di Clinica Medica 3, Viale Morgani, 85, I-50134 Firenze, Italy.

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## References

- Mosmann, T.R., H. Cherwinski, M.W. Bond, M.A. Giedlin, and R.L. Coffman. 1986. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* 136:2348.
- Cherwinski, H.M., J.H. Schumacher, K.D. Brown, and T.R. Mosmann. 1987. Two types of mouse helper T cell clone. III. Further differences in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. *J. Exp. Med.* 166:1229.
- Chang, J.C.C., L. Zhang, T.L. Edgerton, and A.M. Kaplan. 1990. Heterogeneity in direct cytotoxic function of L3T4 cells. Th1 clones express higher cytotoxic activity to antigen-presenting cells than Th2 clones. *J. Immunol.* 145:409.

4. Giedlin, M.A., B.M. Longenecker, and T.R. Mosmann. 1986. Murine T cell clones specific for chicken erythrocyte alloantigen. *Cell. Immunol.* 97:357.
5. Killar, L., G. MacDonald, J. West, A. Woods, and K. Bottomly. 1987. Cloned Ia-restricted T cells that do not produce interleukin-4 (IL-4)/B cell stimulating factor (BSF-1) fail to help antigen-specific B cells. *J. Immunol.* 138:1674.
6. Bottomly, K. 1988. A functional dichotomy in CD4<sup>+</sup> T lymphocytes. *Immunol. Today.* 9:268.
7. Coffman, R.L., B.W. Seymour, D.A. Leberman, D.D. Hiraki, J.A. Christiansen, B. Shrader, H.M. Cherwinsky, H.F. Savelkoul, F.D. Finkelman, M.W. Bond, and T.R. Mosmann. 1988. The role of helper T cell products in mouse B cell differentiation and isotype regulation. *Immunol. Rev.* 102:5.
8. Del Prete, G.F., M. De Carli, C. Mastromauro, R. Biagiotti, D. Macchia, P. Falagiani, M. Ricci, and S. Romagnani. 1991. Purified protein derivative of *Mycobacterium tuberculosis* and excretory-secretory antigen(s) of *Toxocara canis* expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J. Clin. Invest.* 88:346.
9. De Savigny, D.H. 1975. In vitro maintenance of *Toxocara canis* larvae and a simple method for the production of *Toxocara* ES antigen for use in serodiagnostic tests for visceral *Larva migrans*. *J. Parasitol.* 61:781.
10. Parronchi, P., A. Tiri, D. Macchia, M. De Carli, P. Biswas, C. Simonelli, E. Maggi, G.F. Del Prete, M. Ricci, and S. Romagnani. 1990. Noncognate contact-dependent B cell activation can promote IL-4-dependent in vitro human IgE synthesis. *J. Immunol.* 144:2102.
11. Maggi, E., P. Parronchi, G.F. Del Prete, M. Ricci, A. Bosi, L. Moretta, and S. Romagnani. 1986. Frequent T4-positive cells with cytolytic activity in spleens of patients with Hodgkin's disease (a clonal analysis). *J. Immunol.* 136:1516.
12. Romagnani, S., G.F. Del Prete, E. Maggi, G. Bellesi, G. Biti, P.L. Rossi Ferrini, and M. Ricci. 1983. Abnormalities of in vitro immunoglobulin synthesis by peripheral blood lymphocytes from untreated patients with Hodgkin's disease. *J. Clin. Invest.* 71:1375.
13. Del Prete, G.F., E. Maggi, P. Parronchi, I. Chretien, A. Tiri, D. Macchia, M. Ricci, J. Banchereau, J. de Vries, and S. Romagnani. 1988. IL-4 is an essential factor for the IgE synthesis induced in vitro by human T cell clones and their supernatants. *J. Immunol.* 140:4193.
14. Rotteveel, F.T.M., I. Kokkelink, R.A.W. van Lier, B. Kuenen, A. Meager, F. Miedema, and C.J. Lucas. 1988. Clonal analysis of functionally distinct human CD4<sup>+</sup> T cell subsets. *J. Exp. Med.* 168:1659.
15. Coffman, R.L., and J. Carty. 1986. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-g. *J. Immunol.* 136:949.
16. DeKruyff, R.H., S.T. Ju, A.J. Hunt, T.R. Mosmann, and D.T. Umetsu. 1989. Induction of antigen-specific antibody responses in primed and unprimed B cells: functional heterogeneity among Th1 and Th2 T cell clones. *J. Immunol.* 142:2575.
17. Stevens, T.L., A. Bossie, V.M. Saders, R. Fernandez-Botran, R.L. Coffman, R.R. Mosmann, and E.S. Vitetta. 1988. Regulation of antibody isotype secretion by subsets of antigen-specific helper T cells. *Nature (Lond.)* 334:255.
18. Clayberg, C., R.H. DeKruyff, and H. Cantor. 1984. Immunoregulatory activities of autoreactive T cells: an I-A-specific T cell clone mediates both help and suppression of antibody responses. *J. Immunol.* 132:2237.
19. Yasugawa, M., and J.M. Zarlign. 1984. Human cytotoxic T cell clones directed against herpes simplex virus-infected cells. II. Bifunctional clones with cytotoxic and virus-induced proliferative activities exhibit herpes simplex virus type 1- and 2-specific or type common reactivities. *J. Immunol.* 133:2736.
20. Tite, J.P., M.B. Powell, and N.H. Ruddle. 1984. Protein antigen specific Ia-restricted cytolytic T cells: Analysis of frequency, target cell susceptibility, and mechanism of cytolysis. *J. Immunol.* 135:25.