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Manipulation of Th1/Th2 balance in vivo by adoptive transfer of antigen-specific Th1 or Th2 cells

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Abstract

We have investigated the possibility that the Th1/Th2 balance in vivo may be modulated by adoptive transfer of Th1 or Th2 cells induced in vitro. Th1 cells were induced from I-A^d-binding OVA323–339-specific T-cell receptor-transgenic (TCR-Tg) mouse spleen cells by culturing with OVA323–339 peptide and antigen presenting cells (APC) in the presence of IL-2, IL-12 and anti-IL-4 mAb. Th2 cells were induced from TCR-Tg mouse spleen cells by culturing with IL-2, IL-4 and anti-IL-12 mAb in addition to OVA323–339 plus APC. Immunomodulating activities of both Th1 and Th2 cells were determined by their effect on delayed type hypersensitivity (DTH) responses or cytokine production. No significant DTH responses (footpad swelling) were observed in untreated BALB/c mice following a single injection of OVA323–339-pulsed syngeneic spleen cells. However, adoptive transfer of Th1 cells into BALB/c mice induced strong dose dependent DTH responses in response to I-A^d-bound OVA323–339 but not unrelated peptide. In contrast, only slight DTH responses were detected in BALB/c mice transferred with Th2 cells. In parallel with the DTH responses, increased levels of serum IFN-γ were demonstrated in mice adoptively transferred with Th1, while no significant increase was observed in Th2-transferred mice. In vitro analysis also demonstrated that both spleen cells and popliteal lymph node cells prepared from Th1-transferred mice showed Th1-type cytokine production, while cells obtained from Th2-transferred mice revealed Th2-dominant cytokine production. Such immune deviation induced by antigen-specific Th1 cells was demonstrated up to three months after cell transfer. Therefore, it may be possible to manipulate the Th1/Th2 balance in vivo by adoptive transfer of antigen-specific Th1 or Th2 cells. © 1997 Elsevier Science B.V.

Keywords: Th1; Th2; DTH; LFA-1; IFN-γ; IL-4

1. Introduction

T-helper cell populations have been divided into two subpopulations according to their different cy-

Abbreviations: DTH, delayed type hypersensitivity; mAb, monoclonal antibody; TCR-Tg, OVA323–339-specific T cell receptor transgenic

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tokine production patterns (Mosmann and Coffman, 1989; Scott and Kaufmann, 1991). Cells producing interferon-γ (IFN-γ) and IL-2 but no IL-4 are designated as Th1 cells and are believed to be responsible for cellular immunity (Heinzel et al., 1989; Sypek et al., 1993). In contrast, Th2 cells can produce IL-4, IL-5, IL-10 and IL-13 and mediate humoral immunity (Vercelli et al., 1990; Hsieh et al., 1992). It has been suggested that a Th1/Th2 imbalance underlies various immune diseases (Liblau et al., 1995; O'Garra and Murphy, 1995). Examples of Th1-dominant reactions include delayed type hypersensitivity (DTH) responses (Cher and Mosmann, 1987; Magram et al., 1996), contact hypersensitivity (Gautam et al., 1992), experimental autoimmune encephalomyelitis (Kennedy et al., 1992; Martin et al., 1992; Baron et al., 1993) and rheumatoid arthritis (Simon et al., 1994). In contrast, Th2 cells are responsible for atopic diseases and immunoglobulin-mediated autoimmunity (Biancone et al., 1996; Corry et al., 1996).

It has also been demonstrated that there is a genetically controlled difference between mouse strains (especially BALB/c and C57BL/6 mice) in their susceptibilities to Leishmaniasis and Th1-dependent liver injury (Chatelain et al., 1992; Heinzel et al., 1993; Shankar and Titus, 1995; Tanaka et al., 1996). BALB/c mice, which exhibit Th2 dominant immunity, are susceptible to *Leishmania major* infection whereas C57BL/6 mice manifesting Th1-dominant immunity are resistant. Conversely, C57BL/6 mice are sensitive to *Propionibacterium acnes* and LPS-induced Th1-dependent liver injury whereas BALB/c mice are resistant. Thus, the susceptibility of mice to immune disease appears to be affected by the genetically controlled differentiation of T-helper cells into Th1 or Th2 cells.

It is of interest to investigate whether it is possible to manipulate the Th1/Th2 balance in vivo by an appropriate method. In this study, we demonstrate that it is possible to manipulate the Th1/Th2 balance in vivo by cell transfer with antigen-specific Th1- or Th2 cells induced from ovalbumin (OVA)-specific I-A^d-restricted T-cell receptor-transgenic (TCR-Tg) mice in vitro. We also demonstrate that our established DTH model is suitable for investigation of the role of the LFA-1 molecule in the DTH effector phase.

2. Materials and methods

2.1. Animals

Female BALB/c mice were obtained from Charles River Japan (Yokohama, Japan) and used at 5–6 weeks of age. OVA323–339-specific I-A^d-restricted T-cell receptor-transgenic mice, OVA23-3, were established in this laboratory (Sato et al., 1994).

2.2. Cell transfer

Th1 and Th2 cells were induced from OVA23-3 mouse spleen cells. Cells were cultured in RPMI 1640 medium (Gibco BRL, NY) containing 10% fetal calf serum (Filtron Pty., Brooklyn, Australia) with 10 μg/ml of OVA323–339 peptide (Fujiya Co., Hadano, Japan) in the presence of IL-12 (20 U/ml; kindly donated from Genetics Institute, Cambridge, MA) plus anti-IL-4 mAb (50 μg/ml; 11B11, purchased from ATCC, Rockville, MD) for Th1 cells or IL-4 (1 ng/ml; PM-19231W, Pharmingen, San Diego, CA) plus anti-IL-12 mAb (50 μg/ml; C15.1 and C15.6, a kind gift from Dr. G. Trinchieri, Wistar Institute of Anatomy and Biology, Philadelphia, PA) for Th2 cells. Functional differentiation of these cells was confirmed by the development of specific cytokine production patterns using ELISA kits (Amersham International, Buckinghamshire, England).

2.3. Induction of DTH response

Spleen cells from normal BALB/c mice were incubated with OVA323–339 peptide (100 μg/ml)

Table 1
Cytokine production profiles of Th1 cells and Th2 cells induced from OVA23-3 mouse spleen cells

Cells	Stimulation	IFN-γ (pg/ml)	IL-4 (pg/ml)
Th1	—	201	11
	OVA (323–339)	36852	28
Th2	—	84	36
	OVA (323–339)	5541	3474

Th1 cells and Th2 cells were induced as described in Section 2. Cells were restimulated with 10 μg/ml OVA323–339 for 24 h and culture supernatants were collected. IFN-γ levels and IL-4 levels were determined by ELISA.

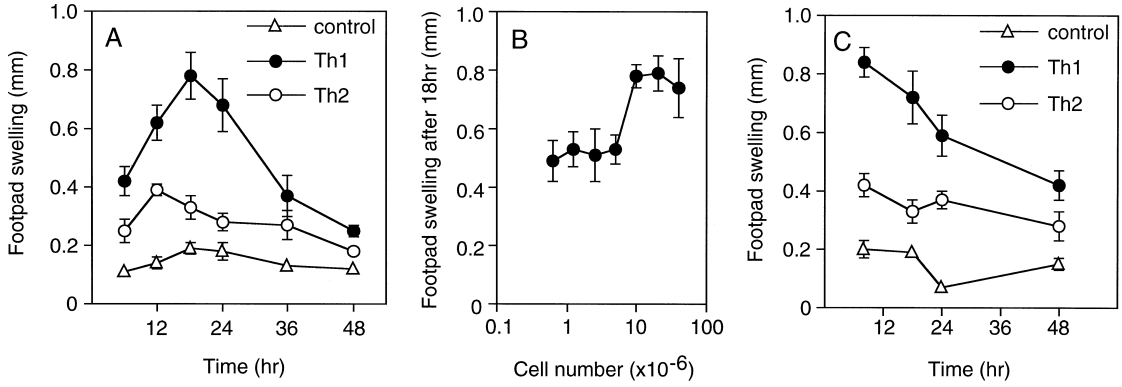

Fig. 1. Induction of primary DTH responses in mice following adoptive transfer of Th1 cells. BALB/c mice, which received cell transfers of Th1 cells (closed circles), Th2 cells (open circles) or neither (open triangles), were primed with 20 μg of OVA323–339. (A) Time-dependent changes in footpad swelling. Footpad swelling was calculated by the following formula; Foodpad swelling (mm) = antigen-injected left hind footpad swelling − untreated right hind footpad swelling. (B) Cell number-dependent DTH induction by Th1 cell transfer. The indicated numbers of cells were transferred into BALB/c mice. Footpad swelling was measured 18 h after antigen challenge. (C) Secondary DTH response in mice transferred with Th1 or Th2 cells. 8 days after the primary challenge, OVA323–339 pulsed syngeneic spleen cells were injected into the left hind foodpad without cell transfer. Foodpad swelling was measured at various times after antigen challenge. The data represent mean ± SE of 5 mice.

at 37°C for 1 h. After washing twice, the cells (10^9) were suspended in 1 ml of PBS including 2 mg/ml of OVA323–339 peptide. As a control peptide, I-A^d-binding PT6 peptide (obtained from Fujiya Co., Hadano, Japan) which was derived from *L. major* cell surface glycoprotein gp63 (Jardim et al., 1990) was used instead of OVA323–339. Then, the cell suspension (10 μl) was injected into the left hind footpad of the mice, which had received cell transfers 4 h previously. Footpad swelling was measured with a digital caliper. Secondary challenge of OVA323–339 peptide was performed by the same protocol 8 days after the primary challenge without cell transfer.

2.4. Histochemistry

Cell migration at the DTH test site was examined after the secondary challenge of antigen. Mice were treated as described above with one modification. Antigen-pulsed spleen cells were replaced by peptide emulsified in Freund's incomplete adjuvant (DIFCO Labs., Detroit, MI) for the secondary challenge, because antigen-pulsed cells may disturb the observation of cell migration. Mice were killed by cervical dislocation and footpads amputated 6 h after the secondary injection were stored in 10% formalin–PBS. Fixed footpads were embedded in paraffin followed by staining with hematoxylin and eosin.

2.5. Cytokine production

Splenic and popliteal lymph node lymphocytes were collected 48 h after the secondary challenge (10

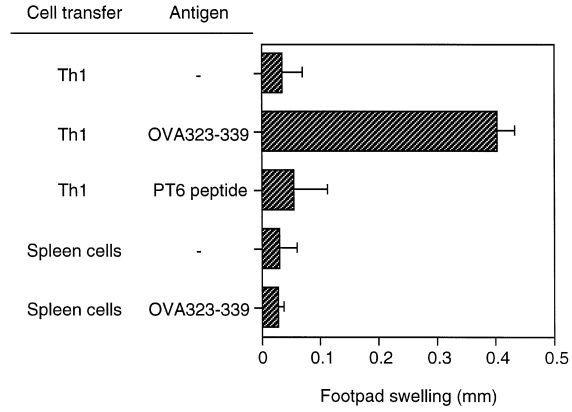

Fig. 2. DTH responses were induced specifically by Th1 cells in response to OVA323–339 peptide. BALB/c mice, which received TCR-Tg mouse-derived Th1 cells or freshly isolated syngeneic spleen cells (2×10^7 cells/mouse), were injected with 20 μg of OVA323–339 or *L. major* PT6 peptide. As a negative control, PBS was injected into footpads (−). Footpad swelling was measured 24 h after the challenge. The data represent mean ± SE of 4 mice.

days after the cell transfer), and were restimulated in vitro with OVA323–339 (10 μg/ml). After 48 h of culture, culture supernatants were tested for IL-4 and IFN-γ levels using ELISA kits from Amersham International (Buckinghamshire, England).

2.6. Blocking of DTH responses by the administration of mAbs

The blocking experiment was performed using an anti-LFA-1 mAb, which was produced in this laboratory (Nishimura et al., 1985). BALB/c mice were injected intraperitoneally (i.p.) with 500 μg/mouse of anti-LFA-1 mAb or, as a control, the same amount of purified rat IgG (Organon Teknika Corp., Durham, NC) 24 and 1 h before the cell transfer.

3. Results

The culture of TCR-Tg mouse spleen cells under Th1-skewing conditions resulted in the generation of an antigen-specific Th1-dominant cell population, producing high levels of IFN-γ but not IL-4 in response to OVA323–339. In contrast, cells cultured using Th2 skewing conditions revealed high levels of IL-4 production though they also produced IFN-γ (Table 1). We used these Th1 or Th2-dominant cell populations for the manipulation of the Th1/Th2 balance in vivo.

The effects of Th1 or Th2 cell transfer on DTH responses were first determined. DTH responses were induced by local administration of OVA323–339-pulsed normal syngeneic BALB/c spleen cells into the footpads of mice pretreated with or without 2×10^7 Th1 or Th2 cells. As shown in Fig. 1A, a single injection of antigen caused no significant DTH induction in mice without cell transfer. However, a striking footpad swelling was observed in mice receiving Th1 cells. Footpad swelling was more prominent in Th1-recipient mice than in Th2-recipient mice which showed only slight swelling. Footpad swelling increased with time after the antigen challenge, reaching a maximum at 18 h and then decreas-

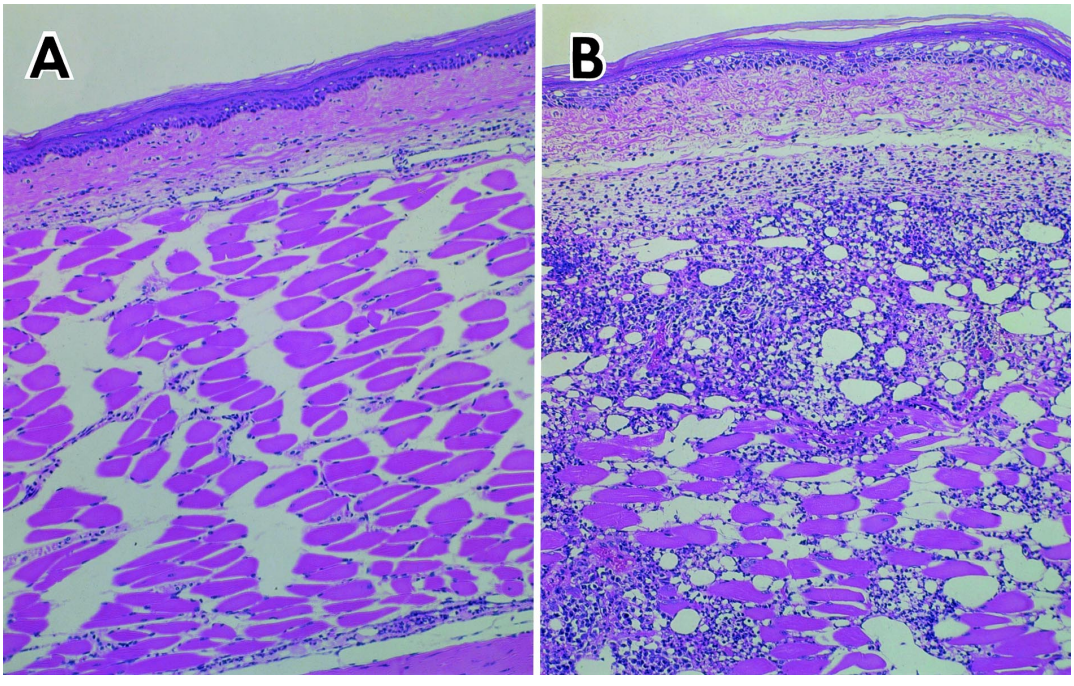

Fig. 3. Cell migration into the DTH test site. Th1-recipient BALB/c mice were primed with syngeneic spleen cells which were pulsed with PT6 peptide (A) or OVA323–339 (B). After 8 days, a secondary challenge was performed by the injection of each peptide emulsified in Freund's incomplete adjuvant. 6 h after the challenge, footpads were sectioned and stained with hematoxylin/eosin.

ing. Furthermore, induction of DTH responses was dependent on the numbers of Th1 cells transferred. As few as 1×10^7 cells/mouse was sufficient to induce maximum swelling at 18 h (Fig. 1B). 8 days after the first immunization, when the footpad swelling had completely vanished, the mice were treated again with the same dose of OVA323–339 peptide antigen without cell transfer. This secondary challenge caused a faster DTH response, which reached a peak at 6 h (Fig. 1C). It was also demonstrated that this DTH response was totally dependent on antigen-specific Th1 cells (Fig. 2). Immunization with unrelated PT6 peptide did not cause significant footpad swelling in mice transferred with TCR-Tg mouse-derived Th1 cells and transfer of syngeneic whole spleen cells failed to induce footpad swelling in response to OVA323–339. Typical DTH responses were also confirmed by histological examination of footpads (Fig. 3). Massive cell migration was observed only in OVA323–339-treated mouse footpads (Fig. 3B), whereas no significant cell migration was observed in mouse footpads challenged with PT6 peptide (Fig. 3A). This suggested that strong antigen-specific DTH responses were induced by Th1 cell transfer, but not by Th2 cells.

We next investigated cytokine production in mice receiving either Th1 or Th2 cells. Consistent with the levels of the DTH responses, the serum IFN-γ levels of mice receiving Th1 cells were markedly elevated 20 h after challenge (Fig. 4). Such significant IFN-γ production in vivo was not detected in mice receiving Th2 cells. Cytokine production patterns in vitro of the spleen and lymph nodes obtained from Th1- or Th2-recipient mice were also examined. After the secondary challenge, spleen cells and popliteal lymph node cells were restimulated with OVA323–339 (10 μg/ml) for 48 h and the cytokine levels (IL-4 and IFN-γ) in the culture supernatants were determined using ELISA kits. As shown in Table 2, both spleen cells and popliteal lymph node cells from Th1-recipient mice produced a great amount of IFN-γ in response to OVA323–339 but no IL-4 was detected. Conversely, cells from Th2-recipient mice produced high levels of IL-4 with some IFN-γ. Both spleen cells and popliteal lymph node cells showed similar

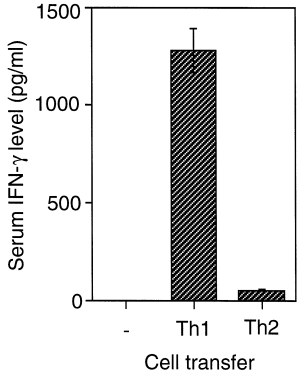

Fig. 4. Elevation of serum IFN-γ levels following antigen challenge in Th1 cell transferred mice. DTH responses were induced by the same method described in the legend to Fig. 1. 24 h after the transfer of Th1 cells, Th2 cells or neither (−) into the mice, serum IFN-γ levels were determined by ELISA. The data represent mean ± SE of 5 mice.

Table 2
Th1- or Th2-dominant cytokine production from popliteal lymph node and spleen cells of BALB/c mice following Th1 or Th2 cell transfer

Cell transfer	IFN-γ (pg/ml)		IL-4 (pg/ml)	
	—	OVA (323–339)	—	OVA (323–339)
(a) Popliteal lymph node				
—	800	800	142	145
Th1	1200	170100	25	47
Th2	300	23450	22	6499
(b) Spleen				
—	100	150	44	33
Th1	500	131600	150	43
Th2	100	31100	61	1371

Following cell transfer (or not), mice were injected in the hind footpad with OVA323–339-pulsed cells at 4 h and 8 days. After an additional 2 days, spleen cells and popliteal lymph node cells were cultured with 10 μg/ml OVA323–339. Supernatants were collected after 48 h of culture for the determination of levels of IFN-γ and IL-4. (a) Popliteal lymph node cells; (b) spleen cells.

Table 3
Cytokine production upon restimulation of spleen cells 3 months after Th1 cell transfer

Stimulation	IFN-γ (pg/ml)	IL-4 (pg/ml)
—	63 ± 4	20 ± 5
OVA (323–339)	12744 ± 2366	37 ± 11

BALB/c mice received 2×10^7 Th1 cells and were then immunized twice with OVA323–339 into the hind footpad as described in the legend to Fig. 1. After 3 months, spleen cells prepared from the mice were restimulated with 10 μg/ml OVA323–339 and supernatants after 48 h of culture were tested for levels of IFN-γ and IL-4 using ELISA.

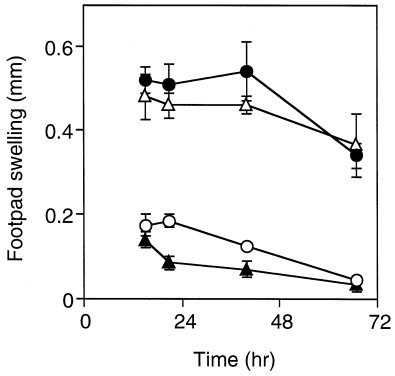

Fig. 5. Inhibition of Th1-induced DTH responses by in vivo administration of anti-LFA-1 mAb. BALB/c mice receiving 5×10^6 Th1 cells were challenged with OVA323–339 antigen as described in the legend to Fig. 1. Anti-LFA-1 mAb (500 μg/mouse) was injected intraperitoneally into mice 24 and 1 h before the cell transfer. Footpad swelling at various times was measured. DTH responses are shown in mice receiving Th1 cells (closed circles), mice treated with anti-LFA-1 mAb and Th1 cell transfer (open circles), mice treated with rat Ig and Th1 cell transfer (open triangles) and in mice without cell transfer (closed triangles). The data represent mean $\pm$ SE of 5 mice.

patterns of cytokine production and their cytokine production profile reflected whether Th1 or Th2 cells were induced (Table 1). No such elevations of IFN-γ and IL-4 levels were observed from OVA323–339-stimulated popliteal lymph node cells from mice receiving 2×10^7 syngeneic whole spleen cells instead (data not shown). Moreover, it was observed that mice receiving Th1 cells showed a similar Th1-dominant cytokine production pattern even after 3 months (Table 3).

As shown in Fig. 5, administration of anti-LFA-1 mAb resulted in a strong inhibition of both DTH responses and IFN-γ production in vivo. This suggests that the LFA-1 molecule is a critical adhesion molecule involved in the DTH effector phase.

4. Discussion

Adoptive immunotherapy using antigen specific CTL or Th cells has been used in the immunotherapy of tumors (Mule et al., 1984; Nishimura et al., 1986, 1992). In the present study, we investigated whether it was possible to manipulate the Th1/Th2 balance in vivo by adoptive transfer of antigen-specific Th1 or Th2 cells. This is of great importance in developing a new strategy for the therapy of immune diseases which are thought to be induced by a Th1/Th2 imbalance. Indeed, it has been demonstrated that IL-12, which activates Th1-dominant immunity, is effective in the treatment of autoimmune diseases induced by an overactivation of Th2 immunity (Donckier et al., 1994; Gavett et al., 1995; Nishimura et al., 1996). Conversely, Th1-dependent immune diseases such as liver injury and graft versus host disease are blocked by suppression of Th1 immunity or activation of Th2 immunity (Racke et al., 1994; Nishimura et al., 1996; Röcken et al., 1996; Tanaka et al., 1996).

There are many factors involved in the regulation of the Th1/Th2 balance such as MHC, antigen, cytokines and costimulators (Murray et al., 1993; Gollob et al., 1995; Hosken et al., 1995; Lenschow et al., 1996). In addition, it has recently been clearly demonstrated that a genetically controlled factor unrelated to the MHC is also involved in the differentiation of Th1 or Th2 cells (Heinzel et al., 1993; Scott et al., 1994; Shankar and Titus, 1995). In an earlier study, we demonstrated that BALB/c mice, which are susceptible to *Leishmania major* infection and resistant to Th1-dependent liver injury, produced high levels of IL-4 in response to anti-CD3 mAb (Nishimura et al., 1997). However, BALB/c mice, which were treated with a cell transfer of OVA323–339 peptide-specific Th1 cells, produced high levels of IFN-γ in response to antigenic peptide in parallel with the elevated DTH responses (Figs. 1–4, Table 2). Such immune responses were not observed in BALB/c mice receiving an adoptive transfer of IL-4 producing Th2 cells having the same antigenic specificity. The immune deviation induced by cell transfer was detected up to 3 months after cell transfer and spleen cells obtained from Th1-recipient mice revealed a Th1-type cytokine production pattern following restimulation with antigen (Table 3). Thus, the finding that Th1 or Th2 cells manipulated in vitro act as long term functional memory Th cells in vivo suggests that adoptive transfer of Th1 or Th2 cells may be a useful tool in immunotherapy. For example, once the antigens involved in autoimmune diseases are characterized, it may be possible to use antigen specific Th1 or Th2 cells in the therapy of autoimmune diseases.

Our established DTH model also makes it possible to investigate the mechanisms of the DTH effector phase. In confirmation of previous results (Cher and Mosmann, 1987), systemically transferred Th1 cells, but not Th2 cells, were found to be the major effector cells inducing DTH responses. Moreover, the LFA-1 molecule was shown to be one of the key adhesion molecules involved in the DTH effector phase (Fig. 5).

We are now investigating various models of immune disease and immune therapy using adoptive transfer of Th1 or Th2 cells.

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