Immunology 1995 **85** 285–291

Occurrence of interleukin-5 production by $CD4^-$ $CD8^-$ (double-negative) T cells in lungs of both normal and congenitally athymic nude mice infected with *Toxocara canis*

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SUMMARY

We studied cells in the lungs of BALB/c and BALB/c-*nu/nu* (nude) mice infected with *Toxocara canis*, which produced interleukin-5 (IL-5) in *in vitro* culture with larval excretory–secretory antigen (ESAg). The proportion of $CD4^+$/$CD8^+$/$CD4^-$ $CD8^-$ cells in lungs of both BALB/c and nude mice was unchanged before and after infection with *T. canis*. Panning and complement-mediated lysis using monoclonal antibody (mAb) to CD4 showed that $CD4^+$ cells in the lung from both mice produced IL-5. Anti-CD4 mAb suppressed ESAg-stimulated IL-5 production *in vitro*. *In vitro* depletion or inhibition of $CD8^+$ cells reduced IL-5 production significantly in some cases, suggesting involvement with IL-5 production. Anti-CD3 mAb enhanced IL-5 production when incubated with or without ESAg. Production of IL-5 was reduced by *in vivo* depletion of $CD4^+$ cells only and both $CD4^+$ and $CD8^+$ T cells, by intraperitoneal injection with appropriate mAb; IL-5 production was stimulated by anti-CD3 mAb. In contrast, IL-5 production by lung cells of BALB/c mice decreased by more than 90% after simultaneous injection with anti-CD4, anti-CD8 and anti-CD3 mAb, and was not enhanced by anti-CD3 mAb. Similar results were obtained in nude mice. These results suggest that $CD4^-$ $CD8^-$ T cells, as well as $CD4^+$ T cells, produce IL-5.

INTRODUCTION

Helminthic infection causes eosinophilia, elevated IgE production and intestinal mastocytosis, which have been suggested to be T-cell dependent in studies using thymectomized or congenitally athymic (nude) animals.[1–4] Recent studies on T-cell derived cytokines have suggested that these immunological responses are all induced by cytokines secreted from the T-helper type-2 (Th2), but not Th1, subset of helper ($CD4^+$) T cells. Eosinophilia was induced by interleukin-5 (IL-5), IgE production by IL-4, and mastocytosis by IL-3 and IL-4.[5] In fact helminthic infections stimulate a T-cell response that has Th2 characteristics.[6–8]

We have focused on the mechanisms of eosinophilia in mice infected with *Toxocara canis*. Infection with *T. canis* causes drastic eosinophilia in the peripheral blood and tissues of the host. Eosinophilia induced after infection with *T. canis* is comparable in athymic (*nu/nu*) and euthymic heterozygous (*nu/+*) mice.[9] Spleen cells from *T. canis*-infected BALB/c mice produce IL-5, as do other helminth-infected mice.[10] In addition, we have demonstrated that lung cells of *T. canis*-infected mice produce a significant level of IL-5 *in vitro* when incubated with larval excretory–secretory antigen (ESAg).[11] In *T. canis*-infected nude mice, lung cells but not spleen cells produce IL-5 *in vitro*.[11] We were interested in this phenomenon and tried to determine what type of cells produce IL-5 in the lung. Augustin *et al.*[12] reported quite numerous T cells with the $CD4^-$ $CD8^-$ double-negative (DN) phenotype, in addition to $CD4^+$ and $CD8^+$ T cells, in the lung of BALB/c mice. The DN population in the lung contains two types of T cells, one bearing $\alpha\beta$ T-cell receptor (TCR), which commonly exists on $CD4^+$ or $CD8^+$ cells, and the other bearing $\gamma\delta$ TCR.[12] We consider that DN cells might produce IL-5, as $CD4^+$ T cells do, since they produce cytokines such as interferon-γ (IFN-γ).[13]

In this study we determined which subset of T cells, namely $CD4^+$, $CD8^+$ or DN cells, is concerned with IL-5 production, by depleting $CD4^+$ and $CD8^+$ cells with *in vitro* and *in vivo* administration of anti-CD4 and anti-CD8 monoclonal antibodies (mAb) from lung cells of both BALB/c and nude mice infected with *T. canis*. Although depletion or inhibition of $CD4^+$ cells reduced IL-5 production, IL-5 production was detectable even after depletion of both $CD4^+$ and $CD8^+$ T cells. These results indicate that DN cells as well as $CD4^+$ cells can produce IL-5.

Received 26 October 1994; revised 31 January 1995; accepted 7 February 1995.

Abbreviations: DN, double-negative ($CD4^-$ $CD8^-$); ESAg, larval excretory–secretory antigen; FITC, fluorescein isothiocyanate; IL, interleukin; mAb, monoclonal antibody; PE, R-phycoerythrin; TCR, T-cell receptor.

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MATERIALS AND METHODS

Mice

Male 8-week-old BALB/cA and BALB/cA-*nu*/*nu* mice were purchased from Clea Japan Inc. (Tokyo, Japan). They were housed in a horizontal laminar flow cabinet and provided with sterile food and water *ad libitum*. At least three mice were used in each experiment.

Parasite and ESAg

Eggs of *T. canis* were collected as described by Oshima.[14] Mice were infected orally with 500 embryonated eggs. ESAg was collected by the method described by de Savigny.[15]

Monoclonal and polyclonal antibodies

Anti-CD4 (GK1.5),[16] anti-CD8α (53-6.72),[17] anti-CD3ε (145-2C11)[18] and anti-IL-5 (NC17)[19] mAb were purified by ammonium sulphate precipitation from ascites from hybridoma-injected mice. Fluorescein isothiocyanate (FITC)-conjugated anti-CD4 (H129.19), and R-phycoerythrin (PE)-conjugated anti-CD4 (YTS 191.1.2) and anti-CD3 (YCD-3-1) mAb were purchased from Gibco BRL (Gaitherburg, MD). FITC-conjugated anti-CD8 (53-6.7) mAb and goat anti-mouse IgG antibody were obtained from PharMingen (San Diego, CA) and Becton Dickinson Co. (Mountain View, CA), respectively. Polyclonal anti-mouse IgG antibody (rabbit) and anti-Thy-1.2 alloantiserum were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Cedarlane (Hornby, Ontario, Canada), respectively.

In vivo *depletion of cells specific for mAb*

Mice were injected intraperitoneally (i.p.) with 2 mg of mAb 3 days before *T. canis* infection. Just before and 7 days after infection, depletion of mAb-specific cells in the peripheral blood of individual mice was examined by flow cytometric analysis, as described below. Lungs were also taken from mice 7 days after infection with *T. canis*, and the lung cells from each mouse were examined by flow cytometry.

In vitro *culture of lung cells*

Mice were anaesthetized and bled from the axillary vein. Lungs were minced and pressed through a stainless steel sieve, and the cells were collected by centrifugation. After washing, 2×10^6 cells were incubated with ESAg (10 μg/ml) in 1 ml of RPMI-1640 medium (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan), containing 10% fetal calf serum (FCS; Biocell Laboratories, Carson, CA) in the presence or absence of mAb (10 μg/ml), for 48 hr at 37° in a humidified atmosphere of 5% CO_2 and 95% air. If necessary, mAb-specific cells were depleted by panning or complement-mediated lysis, as described below, before *in vitro* culture.

Flow cytometric analysis

Single-cell suspensions from the lung or peripheral blood ($0{\cdot}5-1 \times 10^6$ cells) were stained with mAb for 20 min at 4°, after lysing erythrocytes by treatment with cold Ortho-mune lysing reagent (Ortho Diagnostic Systems Inc., Rantan, NJ). Cells were washed three times with cold FACSFlow solution (Becton Dickinson) and analysed by two-colour flow cytometry with a FACScan (Becton Dickinson). The acquired data from 10 000 events were analysed with Lysis II software (Becton Dickinson). Lymphocytes were analysed by gating on forward and side scatter.

Panning

Single-cell suspensions from pooled lungs of BALB/c mice were prepared in Eagle's minimal essential medium (MEM; Nissui). Erythrocytes were removed by centrifugation on Lympholyte-M (Cedarlane). Cells collected from the interface were washed three times with Eagle's MEM, resuspended in cold phosphate-buffered saline (PBS) containing 5% FCS, and panned twice on polystyrene dishes (Greiner, Nurtingen, Germany) coated with antibodies as described by Wysocki & Sato.[20] After resuspension in RPMI-1640 medium, cells were cultured as described above.

Treatment with mAb followed by complement-mediated lysis

Single-cell suspensions (2×10^6) from pooled lungs of BALB/c and nude mice were incubated at 4° for 30 min with 10 μg of mAb and supernatants were removed. Cells were resuspended in RPMI-1640 medium containing 10% rabbit complement (low-toxic; Cedarlane) and 5% heat-inactivated FCS, and incubated at 37° for 45 min. After washing three times with culture medium, cells were cultured as described above.

ELISA to detect IL-5

IL-5 was detected using the sandwich enzyme-linked immunosorbent assay (ELISA) method with anti-IL-5 mAb NC17 and polyclonal goat anti-murine IL-5 antibody (R&D Systems, Minneapolis, MN), as described by Tominaga *et al.*[21] Peroxidase-conjugated anti-goat IgG (rabbit) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS; Wako) were used as a colouring system. The coloured product was measured at a wavelength of 405 nm with a reference wavelength of 490 nm, using a microplate photometer (Corona Electric, Ibaragi, Japan). A standard curve was obtained by using recombinant IL-5 (Genzyme Co., Boston, MA).

Statistical analysis

The statistical significance of the values obtained was evaluated using the Student's *t*-test, and significance was assessed at the $P < 0{\cdot}05$ level of confidence in all cases.

RESULTS

Analysis of T-cell surface markers before and 7 days after infection with *T. canis*

Cells obtained from pooled lungs were panned on dishes coated with antibody against mouse IgG, stained with mAb and analysed using a FACScan. As shown in Fig. 1, the percentages of $CD4^+/CD8^+/CD4^-\ CD8^-$ cells were 62·7/25·1/11·7 (before infection) and 68·6/17·6/13·4 (7 days after infection) in BALB/c mice, and 3·9/4·1/91·8 (before infection) and 3·4/3·4/92·3 (7 days after infection) in nude mice. No significant differences were observed between the percentages before and after infection in both mice.

Panning

Lung cells of BALB/c mice infected with *T. canis* were panned twice on dishes coated with anti-CD4 and anti-CD8 mAb. Only

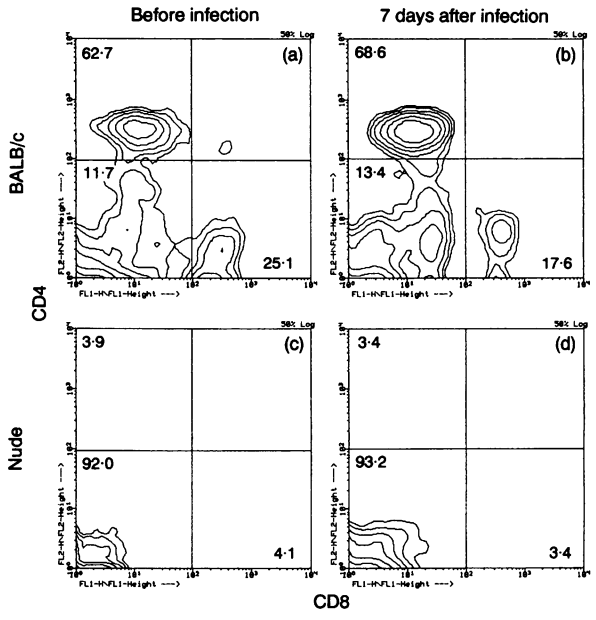

Figure 1. Flow cytometric analysis of lung IgG$^-$ lymphocytes. Lung cells of non-infected (a, c) and *T. canis*-infected (b, d) BALB/c (a, b) and nude (c, d) mice were stained with mAb to CD4 and CD8. Numbers denote percentages of the cells in each area after corrected by subtraction of IgG$^+$ cells from DN cells.

1·4% lymphocytes were CD4$^+$ after panning with anti-CD4 mAb, and only 0·2% were CD8$^+$ after panning with anti-CD8 mAb. IL-5 production was reduced by 63% after panning with anti-CD4 mAb, and by 23% by panning with anti-CD8 mAb (Table 1).

Treatment with mAb followed by complement-mediated lysis

Lung cells of both BALB/c and nude mice infected with *T. canis* were treated with anti-CD4 and anti-CD8 mAb, alone or in combination, followed by incubation with rabbit complement,

Table 1. IL-5 production by lung cells of *T. canis*-infected BALB/c mice after panning with mAb

Panning with	IL-5 produced (U/ml)	Proportion of cells*	
		CD4$^+$	CD8$^+$
—†	993 ± 93	42·0	11·6
Anti-CD4	372 ± 32‡	1·4	12·1
Anti-CD8	762 ± 76‡	42·5	0·2

A single-cell suspension of lung cells was panned twice with dishes coated with anti-CD4 (GK1.5) and anti-CD8 (53-6.72) mAb, followed by *in vitro* culture with ESAg (10 μg/ml) for 48 hr. IL-5 produced in a culture of 2 × 10^6 cells was measured by the sandwich ELISA method. The data are expressed as mean ± SD (n = 4).

* Percentage against total lymphocytes obtained from two-colour flow cytometric analysis.

† Panned with FCS-blocked dishes.

‡ Statistically significant versus panning with FCS-blocked dishes; $P < 0.05$.

Table 2. IL-5 production by lung cells of *T. canis*-infected BALB/c and nude mice after treatment with mAb followed by complement-mediated lysis

Antibody	Complement	IL-5 produced (U/ml)	
		BALB/c	Nude
—	−	515 ± 57	184 ± 10
—	+	466 ± 47 (100)*	192 ± 44 (100)*
Anti-CD4	+	261 ± 33 (56)†	144 ± 36 (75)
Anti-CD8	+	434 ± 54 (93)	162 ± 18 (84)
Anti-CD4 + CD8	+	223 ± 21 (48)†	111 ± 14 (58)†
Anti-Thy-1.2	+	150 ± 17 (32)†	74 ± 6 (39)†

Lung cells (2 × 10^6) were incubated with 10 μg/ml of antibody at 4° for 30 min. After washing, cells were resuspended in RPMI-1640 medium containing 5% heat-inactivated FCS, 10% low-toxic rabbit complement and incubated at 37° for 45 min. After washing three times with medium, cells were then cultured for 48 hr in 1 ml of medium containing ESAg. IL-5 was measured by sandwich ELISA. The data are expressed as mean ± SD (n = 4).

* Calculated as 100%.

† Statistically significant versus treatment with complement alone; $P < 0.05$.

and IL-5 production was compared with control cells treated with complement alone (Table 2). The efficacy of the antibody treatment reached a plateau at 10 μg of each antibody.

CD8-depletion by anti-CD8 mAb and complement treatment did not significantly decrease IL-5 production in either mice, but CD4 depletion with anti-CD4 mAb resulted in about 44% and 25% inhibition of IL-5 production by lung cells from BALB/c and nude mice, respectively. Treatment with both anti-CD4 and anti-CD8 mAb inhibited IL-5 production a little more than did treatment with anti-CD4 alone. The strongest inhibitory effect was observed after treatment with anti-Thy-1 alloantiserum.

Influence of mAb on *in vitro* production of IL-5

Monoclonal antibodies were added to the culture medium and IL-5 production was measured by the sandwich ELISA method. In the presence of ESAg, anti-CD4 mAb inhibited IL-5 production by lung cells by 83% in BALB/c and by 35% in nude mice. Anti-CD8 mAb inhibited it to a smaller but still significant extent in both BALB/c and nude mice. In the absence of ESAg, anti-CD4 mAb did not show any inhibition. On the contrary, anti-CD8 mAb stimulated IL-5 production in both mice. Anti-CD3 mAb stimulated IL-5 production in either the presence or absence of ESAg (Table 3).

In vivo depletion of T cells specific for mAb

An injection of mAb resulted in *in vivo* depletion of cells specific for them from the peripheral blood and lung for at least 10 days (data not shown). Therefore, we examined production of IL-5 by cells collected 10 days after i.p. injection with mAb, followed by *T. canis* infection after an interval of 3 days. To investigate the extent of depletion of mAb-specific T cells, peripheral blood cells and lung cells of individual mice were analysed by flow

Table 3. Influence of co-culture with mAb on *in vitro* production of IL-5 by lung cells of *T. canis*-infected BALB/c and nude mice

mAb	ESAg	IL-5 produced (U/ml) BALB/c	Nude
—	+	4119 ± 186	423 ± 26
Anti-CD4	+	694 ± 48*	277 ± 13*
Anti-CD8	+	2392 ± 142*	347 ± 5*
Anti-CD3	+	9071 ± 452*	1370 ± 42*
—	−	309 ± 17	113 ± 6
Anti-CD4	−	278 ± 13	133 ± 5*
Anti-CD8	−	376 ± 18*	165 ± 6*
Anti-CD3	−	3097 ± 473*	476 ± 35*

A single-cell suspension (2×10^6 cells/ml) of lung cells was incubated with or without combinations of mAb and ESAg (10 μg/ml) for 48 hr. IL-5 production was measured by sandwich ELISA. The data are expressed as mean ± SD ($n = 4$).

* Statistically significant versus culture without mAb; $P < 0.05$.

cytometry. Three days after injection with anti-CD4, anti-CD8 or anti-CD4 plus anti-CD8 mAb, cells specific for them were depleted to less than 0·1% of the total lymphocyte population in peripheral blood of all the mice used in this experiment (Fig. 2b–d). Mice injected simultaneously with anti-CD4, anti-CD8 and anti-CD3 mAb showed depletion of $CD4^+$, $CD8^+$ and $CD3^+$ cells, with a decreased total number of lymphocytes (Fig. 2e). Similar degrees of depletion of mAb-specific cells were observed in the peripheral blood and lung 7 days after *T. canis* infection (Fig. 2g–j, l–o). In addition, the depletion of mAb-specific cells occurred in the profiles of CD4 plus CD8 and CD3. The CD3 expression on lung T cells was lower in extent than that on peripheral blood T cells (data not shown).

IL-5 production by lung cells of mAb-specific cell-depleted mice

Cells from lungs of mAb-treated mice 7 days after infection were pooled and incubated with ESAg for 48 hr, and IL-5 in the culture medium was measured (Table 4). IL-5 produced by the lung cells decreased by about 83% and 24% in BALB/c and nude mice, respectively, with reduced numbers of $CD4^+$ cells compared to the non-treated controls. In contrast, IL-5 production by lung cells from CD8-depleted BALB/c mice increased by 24% compared to the control, accompanied by an increase in the percentage of $CD4^+$ cells against total leucocytes (3·0% and 4·3% in non-treated and CD8-depleted mice, respectively; data not shown). The IL-5 production was nearly the same in CD4- plus CD8-depleted and CD4-depleted BALB/c mice. Cells from CD4-depleted and CD4- plus CD8-depleted mice showed a significant increase in production of IL-5 when incubated with anti-CD3 mAb, suggesting that the non-depleted CD3-bearing lymphocytes with DN phenotype might produce IL-5. Cells from CD4-, CD8- and CD3-depleted

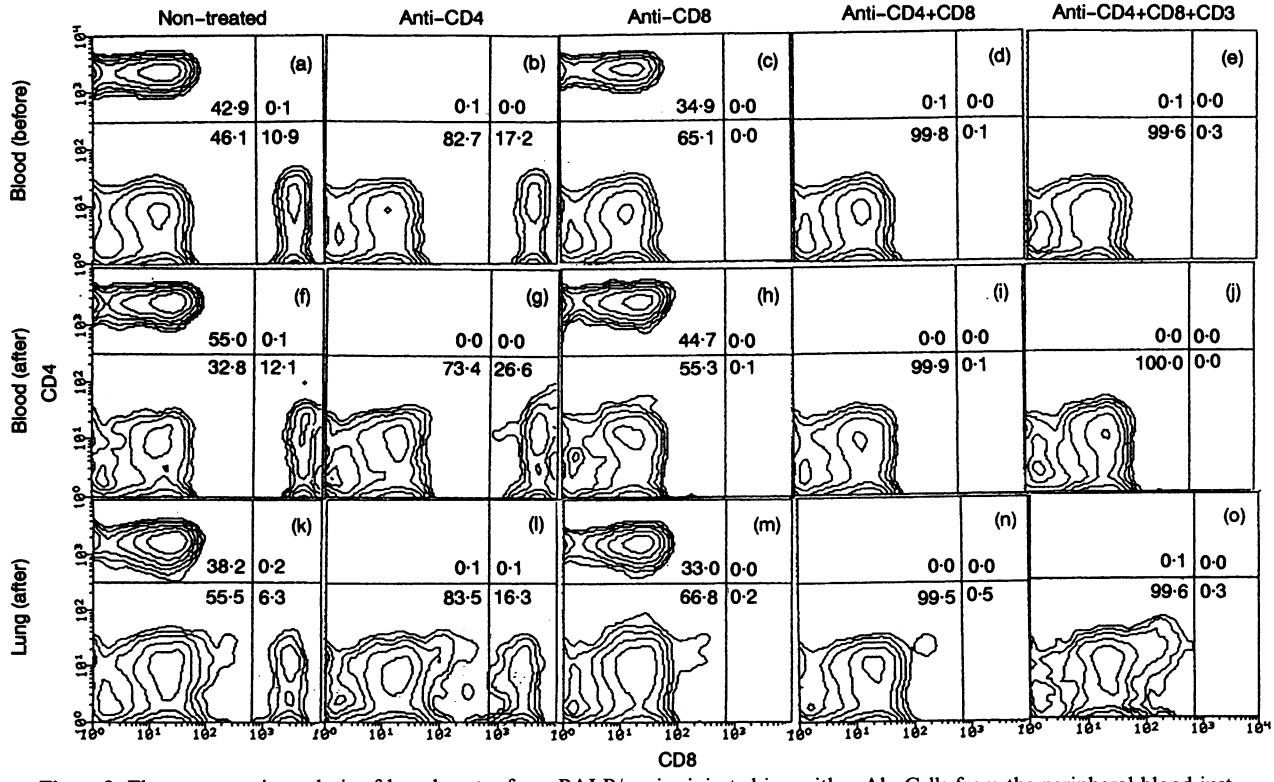

Figure 2. Flow cytometric analysis of lymphocytes from BALB/c mice injected i.p. with mAb. Cells from the peripheral blood just before (a–e) and from the peripheral blood (f–j) and lung (k–o) 7 days after infection with *T. canis* were stained with mAb to CD4 and CD8. Mice were injected i.p. with anti-CD4 (b, g and l), anti-CD8 (c, h and m), anti-CD4 plus anti-CD8 (d, i and n), and anti-CD4, anti-CD8 plus anti-CD3 (e, j and o) mAb 3 days before *T. canis* infection. Data from non-treated mice (a, f and k). Numbers denote the percentages of the cells in each area. Values are means of three mice.

Table 4. IL-5 production by lung cells of *T. canis*-infected mice pretreated with anti-CD4, CD8 and CD3 mAb

	IL-5 produced (U/ml)							
	BALB/c				Nude			
mAb treated	–	4	8	3	–	4	8	3
Non-treated	1369 ± 109	644 ± 119	965 ± 83	3224 ± 159	157 ± 4	107 ± 18	164 ± 8	440 ± 13
Anti-CD4	230 ± 30*	211 ± 53*	257 ± 62*	689 ± 55*	120 ± 4*	134 ± 25	111 ± 16*	196 ± 16*
Anti-CD8	1696 ± 87*	700 ± 11	1368 ± 55*	3795 ± 215*				
Anti-CD4 + CD8	241 ± 17*	254 ± 8*	333 ± 10*	766 ± 65*	74 ± 3*	69 ± 7*	73 ± 6*	145 ± 6*
Anti-CD4 + CD8 + CD3	100 ± 45*	99 ± 36*	93 ± 15*	79 ± 3*†	71 ± 7*	64 ± 13*	70 ± 7*	81 ± 9*†
Non-infected	0*	ND	ND	328 ± 21*				

A single-cell suspension (2×10^6 cells/ml) was incubated with or without mAb in the presence of ESAg (10 μg/ml). The notations –, 4, 8 and 3 denote addition of ESAg only, ESAg with anti-CD4 mAb, anti-CD8 mAb, and anti-CD3 mAb. IL-5 production was measured by sandwich ELISA. The data are expressed as mean ± SD ($n = 4$).

* Statistically significant versus non-treated mice; $P < 0.05$.

† Statistically not significant versus culture without mAb to CD3; $P < 0.05$.

ND, not done.

mice produced a small amount of IL-5 (less than 10% of non-treated mice) and were not stimulated with anti-CD3 mAb. A similar pattern of IL-5 production was observed in nude mice injected with mAb, except for the significant difference between CD4-depleted and CD4- plus CD8-depleted mice (Table 4).

DISCUSSION

We characterized the IL-5-producing cells in the lung of both BALB/c mice and nude mice infected with *T. canis*. Our experimental results indicate two types of IL-5-producing cells, $CD4^+$ T cells and DN T cells. IL-5 production by DN cells has not been reported elsewhere.

We incubated lung cells *in vitro* with ESAg, and IL-5 production in the supernatant was measured by the sandwich ELISA method. Incubation of cells from non-infected mice resulted in no production of IL-5, as checked by the *in vitro* bone marrow culture system,[11] suggesting that the IL-5 production was not due to the mitogenic effects of ESAg.

The treatment of mice with mAb to T-cell surface markers leads to rapid depletion of the mAb-specific subsets of T lymphocytes from the blood and lymphoid tissues, with subsequent immunosuppression of a range of immune functions.[22–26] We applied this to *in vivo* depletion of lymphocyte subsets from the lung. Many investigators have treated mice with less than 1 mg of mAb, resulting in incomplete and transient depletion of appropriate cells. Therefore, we treated mice with higher doses of mAb. Intraperitoneal injection with 2 mg of mAb resulted in the complete depletion of the mAb-specific lymphocytes from the lung and peripheral blood for more than 10 days (data not shown). In addition, *T. canis* infection after an interval of 3 days did not affect the efficiency of mAb (Fig. 2).

In the present study, depletion or inhibition of $CD4^+$ cells resulted in a decrease of ESAg-stimulated production of IL-5, indicating that $CD4^+$ cells produce IL-5. Nevertheless, even when $CD4^+$ cells were reduced to below 0·1% of total lymphocytes, IL-5-producing activity was detected. In addition, depletion of $CD4^+$ T cells had almost no influence on the acute phase of eosinophilia induced by *T. canis* infection, in both BALB/c and nude mice.[27] It is consistent with previous observations that the peak level of eosinophilia in *T. canis*-infected nude mice is as high as that of BALB/c mice,[9] and that the level of IL-5 production by nude lung cells is far lower than that of BALB/c mice (Tables 2, 3 and 4). A reduced production of IL-5 seems to be sufficient for the maximum proliferation of eosinophils. In addition, IL-5 is the only factor that causes eosinophilia in both mice.[27] These results suggest that cells other than $CD4^+$ T cells also produce IL-5.

Depletion or inhibition of $CD8^+$ cells significantly inhibited IL-5 production in some cases. In contrast, co-culture with anti-CD8 in the absence of ESAg resulted in increased production of IL-5. These results suggest that $CD8^+$ cells influence IL-5 production. They might produce IL-5 in the lungs of *T. canis*-infected mice as reported in those infected with *Nippostrongylus brasiliensis*.[28] The increase of IL-5 production by lung cells of CD8-depleted mice compared with non-treated mice may be due to the increased proportion of $CD4^+$ cells.

IL-5 production was not completely suppressed after depletion of both $CD4^+$ and $CD8^+$ cells. IL-5 appeared to be produced by cells bearing the CD3 molecule, since IL-5 production was stimulated by co-culture with anti-CD3 mAb, 145-2C11. In addition, after simultaneous treatment with anti-CD4, anti-CD8 and anti-CD3 mAb, IL-5 production remained at a very low level but was not stimulated by anti-CD3 mAb. Monoclonal antibody 145-2C11 recognizes the ε-subunit of the CD3 complex,[18] and activates T lymphocytes, resulting in cytokine production, cytolysis and proliferation.[18,29,30] In this experiment IL-5 production was demonstrated by co-culture of lung cells from non-infected mice with 145-2C11. These results suggest that DN $CD3^+$ cells may produce IL-5. Svetic *et al.*[8] reported that IL-5 mRNA was expressed by mesenteric lymph node cells from nude and CD4- plus CD8-depleted mice infected with *Heligmosomoides polygyrus*.[8] It is of interest to determine whether these cells are T cells or not.

$CD4^+$ helper T cells were divided into two types, Th1 and Th2 cells, based on their cytokine secretion patterns. Th2 cells produce IL-5 as well as IL-4, IL-6 and IL-10.[31] Th2 cells are

reported to respond to helminthic infections[6–8] and allergic diseases.[32] Infection with *T. canis* causes accumulation of large numbers of eosinophils in the lung. Our data indicate that infection with *T. canis* may activate Th2 cells, followed by production of IL-5 in the lung, and additionally suggest that DN cells may participate in the production of IL-5. Further study of whether these cells are $\gamma\delta$ T cells, $\alpha\beta$ T cells, or both, is now in progress. DN cells have recently been reported to reside in tissues other than the thymus: skin, small intestine, liver, bone marrow and lung.[12,26,33–35] They express mainly $\gamma\delta$ TCR,[12,33,34] and some of them bear $\alpha\beta$ TCR.[12,26,35] They mature extrathymically[34,35] and may contribute to surveillance of epithelium contact with the environment.[36] T cells in the lung were characterized by the low level of CD3 expression on their surface, as reported for T cells in the liver by Tsuchida *et al.*[26] This suggests extrathymic maturation of lung T cells, although we have no evidence concerning the pathway of differentiation to lung T cells and the relation between lung T cells and others which mature extrathymically. Also, it is of great interest to determine whether or not DN cells concern other allergic diseases.

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