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Th2-like response and antitumor effect of anti-interleukin-4 mAb in mice bearing renal cell carcinoma

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Abstract Tumor regression in experimental systems has been linked to the activities of Th1 cells. It is, therefore, conceivable that Th2 cells interrupt the expression of tumor immunity since interleukin-4 (IL-4) and IL-10 inhibit the generation of Th1 from precursors and modulate the competence of antigen-presenting cells to activate this lymphocyte subpopulation. Naive murine renal cell carcinoma (renca) cells (1×10^5) were implanted into the subcapsule of the left kidney of Balb/c and Balb/c nude mice at 6–8 weeks of age. After 14 days, Th2 cytokine (IL-4 and IL-10) mRNAs as well as transforming growth factor β1 mRNA, assessed by reverse transcriptase/polymerase chain reaction were upregulated in the spleen of hosts upon naive renca tumor acceptance, while Th1 cytokine (IL-2 and interferon γ) mRNAs were almost undetectable. In the renca tumor, IL-10 mRNA was detected but IL-2, interferon γ, and IL-4 were not. Intraperitoneal administration of anti-(mouse IL-4) mAb (11B11) reduced the renca tumor size (P = 0.018) and prolonged host survival (P = 0.03), but did not reduce the acceptance rate of the tumor (P = 0.18). However, prior depletion of CD4+ or CD8+ cells with monoclonal antibodies abrogated the antitumor effects of anti-IL-4 mAb. In addition, the significant antitumor effect of anti-IL-4 mAb was not observed in Balb/c nude hosts. Renca cells were transfected with the mammalian expression vector pCAGGS containing murine IL-4 cDNA or vector alone, then stable IL-4 transfectants (RencaL or RencaH, low- or high-IL-4-producing respectively) and control renca cells (RencaC) were obtained. RencaL cells, RencaH cells, or RencaC cells (1×10^5 each) were implanted into the subcapsule of the left kidney of Balb/c, Balb/c nude, and allogenic C3H/HeJ mice, then tumor formation was evaluated 14 days later. When RencaH cells were innoculated into syngeneic Balb/c hosts, tumor volume was marginally suppressed (P = 0.03) and tumors tended to be rejected (P = 0.06) compared with RencaC cells. However, those effects were not observed in Balb/c nude mice. RencaC, RencaL, and RencaH cells were not accepted by allogeneic C3H mice with or without FK506 administration or donor-specific transfusion. The administration of anti-(mouse IL-4) mAb to Balb/c mice significantly suppressed renca tumor growth by a CD4+ and CD8+ T-cell-dependent mechanism. By contrast, relatively high levels of IL-4 production by renca cells and T cells seemed to be required to induce the rejection and growth suppression of IL-4-producing renca cells in syngeneic hosts.

Key words Th1 · Th2 · Cytokine · Anti-IL-4 mAb · Renca

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Introduction

The polar manifestations of the human immune response evolve from expression of the functional programs of lymphocyte subpopulations, Th1 and Th2, which possess coordinate profiles of cytokine release homologous to those identified in the mouse [6]. Tumor regression and allograft rejection in experimental systems have been linked to the activities of T cells with the potential to transcribe interleukin-2 (IL-2) and release interferon γ (IFNγ), for example Th1 [3, 8, 25]. It is, therefore, conceivable that Th2 cells interrupt the expression of tumor immunity since IL-4 and IL-10 inhibit the generation of Th1 from precursors and modulate the competence of antigen-presenting cells to activate this lymphocyte subpopulation [14]. IL-4 and transforming growth factor β1 (TGFβ1) also block cytotoxic T lymphocytes and lymphokine-activated killer cells [12, 17], whereas IL-10 synergizes with IL-4 and TGFβ in inhibiting delayed-type hypersensitivity, and macrophage cytotoxicity respectively [2, 22]. However, tumor cells engineered to release IL-4 induce the regression of established renal cancers as well as other tumors, an observation

that has variously been attributed to leukocyte (eosinophil) recruitment and/or direct effects on tumor growth [9, 20, 26]. We therefore examined the expression of message for prototypic Th1-type (IL-2, IFNγ) and Th2-type (IL-4 and IL-10) cytokines as well as TGFβ1 in the syngeneic murine renal cell carcinoma (renca) model, which is often used as a model of immunological tumor [4], to determine whether the renca tumor is associated with a distinct pattern of cytokine expression.

Materials and methods

Antibodies

Hybridomas producting anti-(mouse IL-4) mAb (11B11), anti-(mouse CD4) mAb (GK 1.5), and anti-(mouse CD8) mAb (53-6.72), purchased from the American Tissue Culture Collection (ATCC), were cultured in RPMI-1640 medium containing 10% fetal calf serum (FCS). Ascites and culture supernatants were collected and antibodies were purified using a Sephadex-Protein G column kit (Pharmacia, Sweden), dialyzed in phosphate-buffered saline, pH 7.4, twice, then filter-sterilized.

Reverse transcriptase/polymerase chain reaction (RT-PCR)/Southern hydridization

RNA was prepared as described [25] from macroscopic tumors in vivo, renca host spleens, and naive renca cells (RencaN, described below) cultured in RPMI-1640 medium supplemented with 10% FCS. Individual aliquots of each sample were resolved in denaturing formaldehyde gels to document the integrity of the RNA (data not shown), essentially as reported [25]. The cDNA was synthesized at 42 °C from 10 μg aliquots of total RNA from tumor or spleen in a total volume of 50 μl buffer supplied with avian myeloblastosis virus reverse transcriptase (RAV-2: Amersham Japan, Tokyo, Japan) and an oligo-dT primer (Promega, Madison, Wis.). The quality of the cDNA was assessed by 35 cycles of PCR amplification of β-actin sequences, as described below, and by examining the products following electrophoresis through 1.5% agarose gels containing ethidium bromide. Primers for β-actin were as described [15]. A 1 μl sample of each cDNA synthesis product was amplified in a thermal cycler (Taitec, Japan) for 40 cycles of denaturation (95 °C for 1 min), annealing (55 °C for 1 min) and extension (72 °C for 1 min). Sense/antisense oligonucleotide primers for IL-2, IL-4 and IL-10 were as described [25] and those for IFNγ and TGFβ1 were 5'-AACTCAAGTGGCATAGATGTGG-3'/5'-CCTGTATTCCGTCTCCTTGG-3' and 5'-CTAATGGTGGACCGCAACAACG-3'/5'-CCTGATTCCGTCTCCTTGG-3' respectively. PCR-amplified cytokine sequences (10 μl) were resolved in a 1.5% agarose gels containing ethidium bromide, then blotted onto Hybond-N (Amersham) in 10×standard saline citrate. The blots were hybridized overnight at 46 °C with midregion oligonucleotides for IL-2, IL-4 and IL-10 in a reference [25] and those for IFNγ and TGFβ1 (5'-ATCAGGCCATCAGCAACAACATAAGCGTCATTGAATCA-7' and 5'-CATTGCTGTCCCGTGCAGAGCTGCGCTTGCAGAGA-3' respectively) labeled with a chemiluminescent residue using a 3'-oligo-labeling kit (Amersham). Blots were then processed according to the manufacturer's instructions and exposed to Hyperfilm (Amersham) for 20–30 min.

Construction of the IL-4 expression vector

Plasmid pUC 18, including 450 bp of synthesized murine IL-4 cDNA, was purchased from BBL (Abington, UK). The insert was excised with *Hind*III and *Bam*HI and blunt-ended with T4 polymerase. After *Eco*RI adapters had been added (Pharmacia) using T4 ligase, the IL-4 cDNA was ligated into the *Eco*RI site of the mammalian expression vector pCAGGS [19], which has a cytomegalovirus (CMV) enhancer and a chicken β-actin promoter. Thus, pCAGGS containing murine IL-4 cDNA in the 5'→3'orientation was constructed (pCIL4-7).

Transfection of murine IL-4 cDNA into renca cells

Plasmid pCIL4-7 was digested with the unique restriction enzymes, *Sal*I or *Sna*BI, to form linear DNA, respectively with/without the influence of CMV enhancer. RencaN cells, which are renca cells cloned by limiting dilution, were co-transfected by electroporation with *Sal*I- or *Sna*BI-digested pCIL4-7 and the neomycin-resistance gene in a molar ratio of 10:1 and cloned in RPMI-1640 medium containing 10% FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin and 400 μg/ml G418.

Direct action of IL-4 on renca cells

To assess the direct action of murine IL-4 on renca cells, 1×10^5 naive renca cells (RencaN) were cultured for 8 days in 10 ml RPMI-1640 medium supplemented with 10% FCS and various concentrations of murine IL-4 (0, 0.5, 5, 50, 500 pg/ml; Fujisawa, Tokyo, Japan).

Mice

Renca cells (RencaN, 1×10^5) were implanted into the subcapsule of the left kidney of 6- to 8-week old Balb/c and Balb/c nude mice (Balb/c nude), purchased from Nisseizai (Tokyo, Japan). After 14 days, Th1 (IL-2 and IFNγ) and Th2 (IL-4 and IL-10) as well as TGFβ1 cytokine mRNA transcripts were assessed by RT-PCR as described above. To assess the effects of anti-IL-4 mAb, 500 μg neutralizing anti-(mouse IL-4) mAb (11B11), or the same amount of control IgG1 (Immunotech, SA) was injected intraperitoneally at the time of implantation with 1×10^5 RencaN cells. Tumor size (calculated by the formula: $V = 0.4w^2l$ (mm^3) was measured 14 days later. Balb/c mouse survival was also evaluated after innoculation with 1×10^5 RencaN cells with/without the intraperitoneal administration of 500 μg 11B11. To some Balb/c mice, 1 mg depleting anti-(mouse CD4) (GK 1.5) or 400 μg depleting anti-(mouse CD8) mAb (53–6.72) was administered 24 h before RencaN cell implantation with/without anti-IL-4 mAb (11B11). The intraperitoneal administration of GK 1.5 and 53-6.72 at these dosages respectively reduced $CD4^+$ and $CD8^+$ cells in splenocytes, as assessed by flow cytometry (EPICS-CS), to less than 2% ($n = 2$ in each case) 24 h later. RencaL (a low-IL-4-expressing renca clone: RenlSal) and RencaH (a high-IL-4-expressing renca clone: RenlSnl) cells, which are described in Results (IL-4 transfection into renca cells and its effects), or RencaC (transfected with a vector alone) cells (1×10^5 each) were implanted into the subcapsule of the left kidney of Balb/c, Balb/c nude, and C3H/HeJ mice; tumor formation was evaluated 14 days later. FK506 (3 mg/kg; provided by Fujisawa Pharmaceutical Company) was administered to some C3H hosts intramuscularly for 5 consecutive days from the day before RencaC, RencaL, RencaH cell implantation. Heparinized whole donor-strain (Balb/c) blood (0.3 ml) was transfused into the penile vein 9 days before implantation of those cells into other C3H hosts.

Histology

Cryostat specimens (renca tumor and recipient spleens) were fixed in acetone for 3 min at −20 °C and incubated with 3% H_2O_2 then with anti-(mouse CD4) rat mAb (L3T4; 5H10-1), anti-(mouse CD8) rat mAb (Lyt-2; RM4-5), or anti-(mouse asialo-GM1) (NK marker) rabbit mAb for 1 h at room temperature. After several rinses with phosphate-buffered saline (pH 7.4), specimens were incubated with biotinylated anti-(rat IgG) or anti-(rabbit IgG) for 20 min, washed with phosphate-buffered saline, then incubated with avidin-biotin-peroxidase complex (Vector Laboratory, Calif.). The specimens were immersed for 5 min in 0.5% 3.3'-diaminobenzidine with 0.005% H_2O_2 to visualize the immunoreactive sites. The specimens were then counter-stained with hematoxylin for 1 min.

Table 1 Tumor acceptance and growth. *RencaN* naive renca cells, *RencaC* RencaN cells transfected with vector alone, *RencaL* low-IL-4-producing RencaN cells, *RencaH* high-IL-4-producing RencaN cells

Host	Tumor	Treatment	*n*	Tumor acceptance (%)	*P*	$\log_{10}(1+V)$ (mean ± SE)	*P*
Balb/c	RencaN	Control IgG1	9	100*1		1.89±0.22*1	
	RencaN	Anti-IL-4 mAb	7	77.8	0.18 to *1	0.38±0.18	0.018 to *1
	RencaN	Anti-CD4 mAb + control IgG1	7	85.7	0.47 to *1	1.82±0.48 (NS)	>0.99 to *1
	RencaN	Anti-CD4 mAb + anti-IL-4 mAb	6	100	>0.99 to *1	2.36±0.22 (NS)	0.92 to *1
	RencaN	Anti-CD8 mAb + control IgG1	6	100	>0.99 to *1	2.43±0.17 (NS)	0.87 to *1
	RencaN	Anti-CD8 mAb + anti-IL-4 mAb	5	100	>0.99 to *1	1.77±0.34 (NS)	>0.99 to *1
Balb/c nude	RencaN	Control IgG1	6	100*2		1.78±0.45*2	
	RencaN	Anti-IL-4 mAb	6	83.3	>0.99 to *2	1.62±0.45	0.81 to *2
Balb/c	RencaC	None	9	100*3		1.99±0.20*3	
	RencaL	None	5	100	>0.99 to *3	1.54±0.13	0.52 to *3
	RencaH	None	7	57.1	0.06 to *3	0.92±0.36	0.03 to *3
Balb/c nude	RencaC	None	6	100*4		1.82±0.40*4	
	RencaH	None	6	83.3	>0.99 to *4	1.15±0.42	0.27 to *4

*1–*4 Statistical comparisons. *NS* not significant ($P > 0.05$)

Statistics

Analysis of variance (ANOVA; Scheffe test) or the unpaired *t*-test was applied to compare the logarithms of tumor volume [$\log_{10}(1+V)$]. Fisher's Exact test was applied to compare the tumor acceptance rate.

Results

Direct action of IL-4 on renca cells

RencaN cell growth and morphological figures (data not shown) were not directly influenced by murine IL-4 added to the culture [cell numbers in cultures: 42.3×4.0 (mean±SD), 40.1±3.5, 39.2±3.8, 39.5±4.2, 34.8±3.8×10^5 cells for 0, 0.5, 5, 50, 500 pg/ml IL-4 respectively, $n = 2$].

Naive renca tumor formation in mice

As shown in Table 1, naive renca cells (RencaN) were accepted by all of Balb/c and Balb/c nude mice. The RencaN cell tumor size in Balb/c was not statistically different from that in Balb/c nude ($P = 0.81$, unpaired *t*-test).

Cytokine mRNA expression in the naive renca tumor and in the spleens of host Balb/c

Th1-type (IL-2 and IFNγ) and Th2-type (IL-4 and IL-10) as well as TGFβ1 cytokine transcripts were assessed by RT-PCR. As shown in Fig. 1, naive renca cells (RencaN), cultured in vitro, expressed TGFβ1 mRNA. However, Th1-type (IL-2 and IFNγ) or Th2-type (IL-4 and IL-10) cytokines were not expressed. As shown in Fig. 2, in the

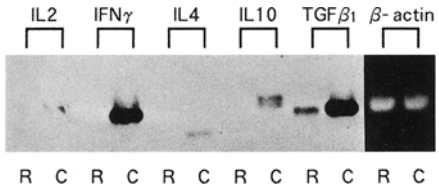

Fig. 1 Th1/Th2 cytokine and transforming growth factor β1 (*TGFβ1*) mRNA expression in naive renal cell carcinoma (renca) cells (RencaN) cultured in vitro. *R* native renca (RencaN) cells. *C* positive control (a sample of a cardiac allograft from a mouse suffering from allograft rejection)

rencaN tumor of the left kidney of Balb/c mice, neither IL-2 nor IFNγ mRNA was expressed. IL-10 mRNA as well as TGFβ1 was expressed, while IL-4 mRNA was not. In the spleen of rencaN (naive renca cells) tumor-bearing Balb/c mice, Th2-type cytokine mRNAs (IL-4 and IL-10) as well as TGFβ1 mRNA seemed upregulated during naive renca tumor acceptance, while Th1-type cytokine mRNAs were almost undetectable: IL-2 was not expressed and IFNγ was marginally expressed in the spleen of rencaN tumor-bearing Balb/c hosts, except for one intense IFNγ signal in a mouse whose tumor was relatively small. None of the tested cytokines was detected in the naive Balb/c spleen. β-actin mRNA was found in all samples tested as shown.

Effects of anti-IL-4 mAb administration on growth of renca tumor

As shown in Table 1, neutralizing anti-(mouse IL-4) mAb caused an antitumor effect in Balb/c by reducing renca tumor size ($P = 0.018$), although the reduction in tumor acceptance rate was not significant ($P = 0.18$). Renca tumor host survival was marginally, but significantly prolonged by

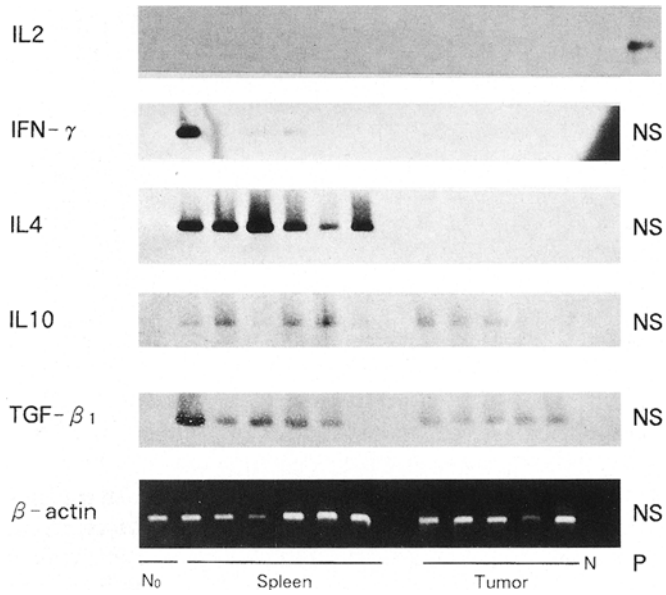

Fig. 2 Thl/Th2 cytokine and TGFβ1 mRNA expression in the renca tumor and in the spleen of hosts. No spleen of naive Balb/c mouse; *N* negative control (no cDNA in polymerase chain reaction); *P* positive control for interleukin-2 (IL-2) (a sample of a cardiac allograft from a mouse suffering from allograft rejection), *NS* no sample, IFNγ interferon γ

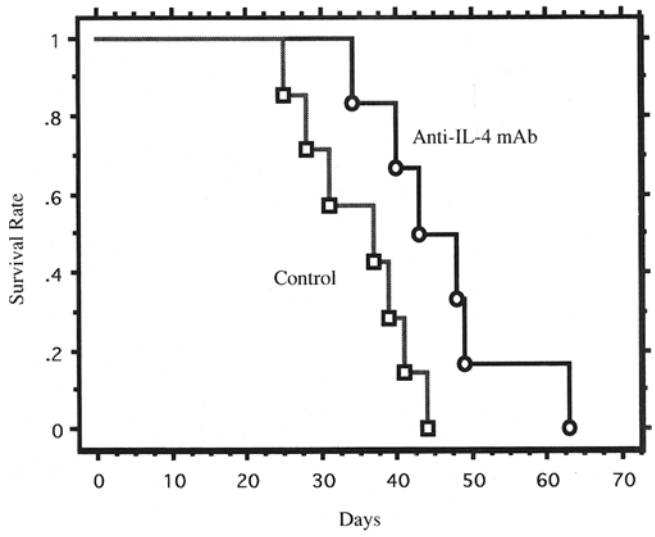

Fig. 3 Survival of host Balb/c mice inoculated with naive renca (RencaN) cells ($p = 0.03$). *Control* control mice given control IgG1, *Anti-IL-4 mAb* mice administered 500μg anti-IL-4 mAb (11B11)

the anti-(mouse IL-4) mAb (46.2±4.0 days: mean±SE) compared with control animals (35.0±2.7 days) given IgG1 ($P = 0.03$, log-rank test, Fig. 3). However, the prior depletion of CD4+ or CD8+ cells in Balb/c hosts abrogated the antitumor effects of anti-IL-4 mAb.

In addition, the antitumor effects of anti-IL-4 mAb was not observed in Balb/c nude hosts. Immunohistologically, CD4+ and CD8+ cells in renca tumors were observed both in control Balb/c recipients of renca tumor and in renca recipients given anti-(mouse IL-4) mAb (Fig. 4A). Anti-asialo GM1 mAb, detecting natural killer (NK) cells, failed to find NK cells in the renca tumor, because it also stained renca cells themselves (data not shown). CD4+ and CD8+ cells were detected in the germ center in recipient spleens of the two groups as well as in the normal Balb/c spleen (Fig. 4B), while NK cells were observed more in the para-germ center region. Notably, Balb/c hosts given anti-(IL-4) mAb, which then rejected the tumor, showed marked splenomegaly with prominent multinucleated giant cells (Fig. 5). Balb/c mice administered 500 μg anti-IL-4 mAb alone (without renca cells) did not develop splenomegaly and multinucleated giant cell formation ($n = 3$, data not shown).

IL-4 transfection into renca cells and its effects

We isolated the renca clones, Ren1Sal (transfected with *Sal*I-digested pCIL4-7, with CMV enhancer influence) and Ren1Sn1 and Ren1Sn14 (transfected with *Sna*BI-digested pCIL4-7, without CMV enhancer influence). A culture of 1×10^5 Ren1Sal (RencaL) in RPMI-1640 medium containing 10% FCS produced 185 pg murine IL-2/ml in 24 h, measured with an IL-4 enzyme-linked immunosorbent assay kit (Endogen, Boston, Mass.), in the culture supernatant, while 1×10^5 Ren1Sn1 (RencaH) and Ren1Sn4 cells produced 1682 pg/ml and 1287 pg/ml in 24 h respectively. Control RencaC cells (1×10^5) produced 27 pg/ml in 24 h (background).

As shown in Table 1, renca cells (RencaH) producing relatively high amounts of IL-4 tended to be rejected (in 42.9% of Balb/c hosts), while 100% of the cells producing relatively low amounts of IL-4 (RencaL) or control renca (RencaC) cells were accepted (for RencaH versus RencaC, $P = 0.06$ by Fisher's Exact test) by Balb/c hosts. Tumor volume suppression was also marginally significant when RencaH cells were inoculated, compared with RencaC cells ($P = 0.03$), while RencaL cells did not cause tumor volume suppression ($P = 0.52$). Implantation of RencaH cells in Balb/c nude hosts abrogated this tendency towards tumor rejection ($P > 0.99$ for RencaH versus RencaC by Fisher's Exact test) and tumor volume suppression. ($P = 0.27$ for RencaH versus RencaC, unpaired *t*-test). Immunohistologically, CD4+ cells and CD8+ cells were rarely detected in RencaH tumors (Fig. 4A) compared with RencaC tumors. CD4+ cells, CD8+ cells, and NK cells were found in spleens of recipients of RencaH and RencaC tumors (Fig. 4B). In allogeneic C3H mice, control renca (RencaC, RencaH, and RencaL were not accepted with or without FK506 or donor-specific transfusion ($n = 5$ for each group).

Discussion and conclusions

Although the cellular origins of cytokine transcripts detected in the renca tumor and the recipient spleen cannot be defined by our approach, the data are consistent with the differential expression of Th2-type cytokine mRNAs as

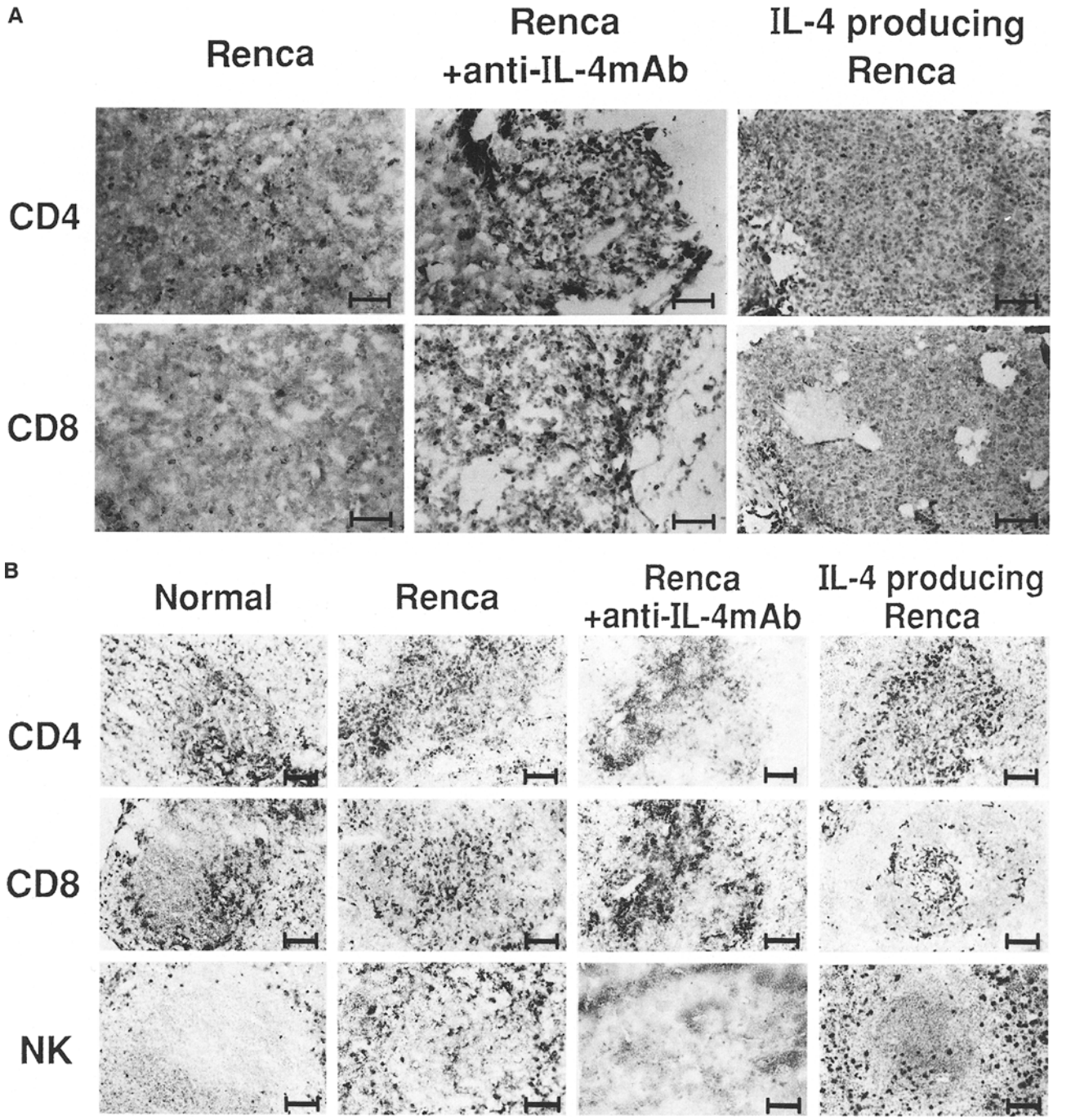

Fig. 4 A Immunohistochemical staining of CD4+ cells and CD8+ cells in renca tumor. *Renca+anti-IL-4* RencaN tumor in Balb/c mice given 500μg 11B11, *IL-4 producing Renca* RencaH tumor. *Bar* 100 μm **B** Immunohistochemical staining of CD4+, CD8+, and natural killer (NK) cells in spleens of renca hosts. *Normal* normal Balb/c spleen, *Renca*, sleen of Balb/c mouse bearing RencaN tumor, *Renca+anti-IL-4 mAb*, spleen of Balb/c mouse bearing RecaN tumor given 500μg 11B11, *IL-4 producting Renca,* spleen of Balb/c mouse bearing tumor. *Bar* 100 μm

well as TGFβ1 mRNA in the spleen of renca-bearing mice. It is possible that inflammatory and infiltrating cells other than T cells (e.g. NK cells [11]) in the tumor and tumor cells themselves transcribe cytokine mRNAs. However, naive renca cells did not express Th1-type and Th2-type cytokine mRNA. A defect in IL-2 and IL-4 mRNAs and the selective expression of IL-10 mRNA in human renal tumor have been described [18] suggesting that they contribute to the impaired immunity that often arises in cancer patients. IL-2 and IL-4 mRNAs as well as IFNγ mRNA were consistently undetectable in the renca tumor while IL-10 mRNA was present, suggesting that Th1- and Th2-like T cells were not activated in the tumor. IL-10 down-regulates MHC class II expression on monocytes, leading to impaired

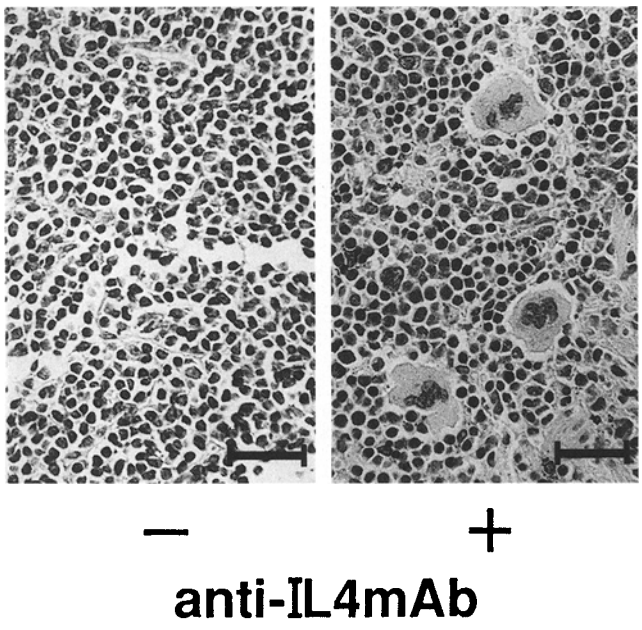

Fig. 5 Histopathology (hematoxylin/eosin staining) of the spleen of Balb/c hosts inoculated with RencaN cells. *Left* administered control IgG1; *right* administered anti-IL-4 mAb and no macroscopic tumor at 14 days. Numerous multicleate giant cells were observed upon the administration of anti-(mouse IL-4) mAb. Bar 60 μm

antigen-presenting ability [7], directly inhibits the growth of T cells [23], and suppresses the production of IFNγ by CD4+ Th1 cells [16]. IL-10 in renca tumor could have been produced by non-T cells such as macrophages and B cells [7, 21] as its expression was not concurrent with that of IL-4. In the spleen of renca-bearing hosts, marginal IFNγ mRNA expression that was not concurrent with IL-2 could have been transcribed by NK cells [11].

IL-4, an autocrine growth factor of Th2 cells, may promote biased differentiation of CD4+Th0 into CD4+Th2 after tumor implantation and maintain renca tumor growth in syngeneic Balb/c hosts. The administration of the anti-(mouse IL-4) mAb at the time of renca cell inoculation into Balb/c mice significantly suppressed renca tumor growth, but did not necessarily cause tumor rejection. Anti-IL-4 mAb also prolonged renca tumor host survival. However, the prior depletion of CD4+ or CD8+ T lymphocytes abrogated the antitumor effect of anti-IL-4 mAb. In addition, the administration of anti-IL-4 mAb in Balb/c nude hosts did not suppress renca growth. Thus, both CD4+ and CD8+ cells are necessary to induce the antitumor effects of anti-IL-4 mAb so activation of Th2-type cytokines and tumor suppression induced by anti-IL-4 mAb in renca hosts may be linked to the activities of T cells. Anti-IL-4 mAb administration might have suppressed renca tumor growth by blocking differential CD4+ Th2-like cell activation and, presumably, by biasing the differentiation of tumor-reactive CD4+Th0 into CD4+Th1 cells in hosts, augumenting cell-mediated immunity. Immunohistological staining detected CD4+ and CD8+ as well as NK cells at comparable levels in control hosts and hosts given anti-IL-4 mAb. However, this may merely imply that lymphocyte phenotypes do not indicate their functions. It is conceivable that dendritic cells bearing processed tumor-specific antigens migrate to the host spleen [13] and that a host immune response initiated at this level (tumor-specific cytotoxic T lymphocytes) modulates tumor growth. Therefore, localized tumor-induced splenic effects could be related to tumor growth and splenic cytokine mRNA expression may not necessarily be the same as that at the tumor site. Takashima et al. reported that the suppression of concanavlin-A-induced multinucleated giant cell formation in monocyte culture by IL-4, antagonizing the enhancing effect of IFNγ, was completely abrogated by anti-IL-4 mAb [24]. Multinucleated giant cell formation in the present study may be a similar event to this in vivo.

While tumor cells engineered to release IL-4 induce the regression of established renal cancers by a non-T mechanism [9, 20], we postulated that lower levels of IL-4 produced by tumor cells may differentially activate Th2-like cells, suppress Th1-like cells by releasing Th2-type cytokines, and induce tumor growth even in allogeneic hosts. To test this, we established murine IL-4-transfected renca clones producing relatively high or low amounts of murine IL-4 protein and assessed their growth in syngeneic (Balb/c, H-2d) and allogeneic (C3H/HeJ, H-2k) hosts (Table 1). Renca cells that were high-IL-4 producers tended to be rejected by syngeneic Balb/c hosts ($P = 0.06$), while 100% of the cells producing low amounts of IL-4 or naive renca cells were accepted.

In addition, the growth of high-, but not low-IL-4-producing renca cells was marginally, but significantly, suppressed compared with that of naive renca cells ($P = 0.03$). However, this tendency for renca rejection and growth suppression was not found in Balb/c nude mice ($P > 0.99$ and $P = 0.27$ respectively). Thus, the relatively high level of IL-4 production by renca cells and T cells seemed necessary to induce the rejection and growth suppression of IL-4-producing renca cells in syngeneic hosts. CD4+ and CD8+ cells were hardly observed inside the RencaH tumor. T cells may be necessary to induce IL-4-induced tumor suppression but effectors seem to be non-T cells as described [26]. Engineered IL-4 production by renca cells implanted in allogeneic C3H hosts did not cause tumor acceptance, even with the help of immunosuppressive regimens, contrary to what we had expected.

The biological significance of TGFβ1 expression in murine/human RCC is unclear. TGFβ1 inhibits immediate and delayed-type hypersensitivity and synergizes with IL-10 to inhibit macrophage activation. This factor, therefore, can modulate immune and inflammatory responses. Progressive immunoincompetence and concomitant immunity in the tumor-bearing hosts [1, 5] might be explained by the Th1, Th2 paradigm as appears to be true of other model systems [10, 25].

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