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ORIGINAL PAPER

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Simultaneous ligation of CD5 and CD28 with monoclonal antibodies restores impaired immunostimulatory function in human renal cell carcinoma

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Abstract Tumor cells, including renal cell carcinoma (RCC) cells, do not effectively stimulate T lymphocyte responses against specific antigens presented on their surface. Reasons for this low immunogenicity may include low or absent expression of MHC class I and/or class II molecules, as well as accessory and costimulatory molecules. We used tumor cell pretreatment with cytokines, together with monoclonal antibodies (mAbs) directed at receptors for costimulatory molecules, to render RCC cells immunostimulatory. Interferon-γ or tumor necrosis factor-α pretreatment enhanced expression of MHC class I and class II molecules, as well as CD54, but had only minimal effects on T cell activation. A CD28 mAb, or an even more effective combination of CD28 and CD5 mAb, induced strong primary proliferative responses of allogeneic resting T lymphocytes. Cytokine pretreatment further augmented this T cell response in vitro and allowed T cell expansion and establishment of T cell lines. Stimulation of T cells with autologous RCC cells resulted in a similar T cell activation but with the expansion of cytolytic T cells directed at autologous MHC class II molecules. These experiments demonstrate that cytokines combined with costimulatory mAbs are useful for increasing the immunogenicity of tumor cells. They also indicate, however, that autologous MHC class II expression on tumor cells, together with strong costimulation, may lead to the activation of autoreactive T cells.

Keywords Tumor immunology · Renal cell cancer · CD5 and CD28 · Costimulation · Autoimmunity

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Introduction

Spontaneous regression and partial or complete remission of metastatic renal cell carcinoma (RCC) and melanoma in some patients after immunotherapy provided the first indications for the presence of cellular antitumor immune responses [33]. In addition, RCC, melanoma, and some other solid tumors frequently contain CD3+ rich T lymphocyte infiltrates that often display antitumor reactivity when cultured in vitro, suggesting a participation of T lymphocytes in human tumor immunity [1, 12, 22, 24, 25, 37].

Binding of the T cell receptor (TCR) to an MHC/antigen (Ag) complex provides the first signal for T cell activation and determines the specificity of a T cell response [38]. Additional interactions between accessory molecules, such as CD54, CD58, CD72, and CD80/CD86, and their corresponding receptors CD11/18, CD2, CD5, and CD28/CTLA-4, deliver costimulatory second signals [31]. Only the combination of Ag-specific and costimulatory signals leads to complete T lymphocyte activation, clonal expansion, and differentiation of lymphocytes into effector T cells [38], as well as para- and autocrine cytokine interactions. Stimulation of the TCR in the absence of costimulatory signals induces antigen-specific T cell anergy [19, 28].

Recently, in the case of melanoma, numerous tumor-associated antigens (TAAs) have been identified at the molecular level [3]. TAA from most other cancers are less well characterized, although evidence for RCC-derived T cell antigens has been reported [4, 5, 13, 17, 18, 23, 32]. The low expression of TAAs may be one reason

that inefficient immune responses are generated against RCC. Another cause for the low immunogenicity of tumor cells may be their failure to express costimulatory molecules such as CD80 and CD86. Consequently, immune cells encountering tumor cells may receive signal 1 in the absence of signal 2, resulting in the induction of T cell anergy [31, 38]. Furthermore, downregulation of accessory molecules such as CD58 and CD54 and MHC class I molecules, as well as lack of MHC class II expression, may contribute to immune escape [29, 34, 39]. As a compensation, providing costimulatory signals represents one strategy to augment the immunogenicity of tumor cells and to activate tumor-specific T lymphocytes. Since many autologous antigens are presented by tumor cells, increasing the immunogenicity of tumor cells harbors the potential of not only inducing tumor-specific responses but also of breaking tolerance against autoantigens.

Costimulatory signals can be provided through genetic modification of tumor cells, allowing them to express cytokines and/or costimulatory molecules such as CD80. Such modified tumor cells were demonstrated to induce tumor-specific immune responses and tumor protection in animal models [2, 7, 10, 11, 14, 16, 41, 44]. In the case of human tumor cells, CD80 expression was shown in vitro to render tumor cells capable of inducing resting T lymphocyte proliferation and differentiation into cytolytic effector cells [20].

The CD5 receptor on T lymphocytes is known to be involved in T cell activation and T–B cell interactions. Recently it could be shown that simultaneous ligation of CD5 and CD28 could induce T cell activation in the absence of signal 1, providing evidence for a pathway of Ag-independent T cell activation via CD5 and CD28 [46]. This pathway of activation may be interesting in cases of tumor cells where specific tumor antigens are unknown.

The present report describes an alternative strategy for providing costimulatory signals. Cytokine pretreatment of tumor cells was employed to augment the expression of cell surface molecules in combination with monoclonal antibodies (mAbs) directed against the T cell molecules CD28 and CD5 in order to provide costimulatory signals. We demonstrate that such an approach produced activated T cells directed against nonimmunogenic RCC cells previously unable to induce T cell responses. However, our studies also indicate that such treatments contain the risk of activating autoreactive T cells.

Materials and methods

Cell lines

The human RCC cell lines KTCTL-30, -104, -140, -187, and -195 were obtained from the DKFZ Tumorbank (Heidelberg, Germany). All cell lines were grown as adherent monolayers in tissue culture flasks in RPMI medium (Gibco, Eggenstein, Germany) supplemented with 10% FCS (Serva, Heidelberg, Germany), 2 mM L-glutamine (Gibco), and 1% penicillin/streptomycin (Gibco). Confluent cultures were passaged using 10 mM EDTA in phosphate-buffered saline without Ca2+ and Mg2+, and 0.05% trypsin-EDTA (Gibco). Peripheral blood mononuclear cells (PBMC) were prepared from peripheral blood of healthy donors or – in two cases (KTCTL-187, -195), from patients with RCC – by density gradient centrifugation using Ficoll (Ficoll-Hypaque, Farmacia, Uppsala, Sweden). MHC class II typing using specific primers for PCR confirmed that T cells and tumor cell lines originated from the same patient (data not shown). T lymphocytes were subsequently prepared by adherence of PBMC on plastic dishes followed by rosetting with sheep erythrocytes (ICN, Meckenheim, Germany). T cells were >90% CD3+, >95% CD2+, and <1% CD14+, as determined by flow cytometry. M7 is an Epstein-Barr virus-transformed lymphoblastoid cell line (B-LCL). K562 is a natural killer (NK) cell sensitive erythroleukemic cell line. SkMel63 is a melanoma cell line.

Immunofluorescence

Indirect immunfluorescence was performed as described [30]. MAbs were anti-MHC class I (W6/32, ATCC), anti-MHC class II (AIMHCII, our laboratory), anti-CD54 (MEM112, Dr V. Horejsi, Prague, Czech Republic), anti-CD58 (PAK1, our laboratory), anti-CD80 (clone 104, 1 mg/ml; Dianova, Hamburg, Germany), and anti-CD86 (IT2.2, 2 mg/ml; Dianova, Hamburg, Germany). As a secondary antibody, fluoresceinated rabbit antimouse-Ig (F(ab′)2) (Dako, Hamburg, Germany) diluted 1/40 in culture medium, was used. Negative control samples were incubated with the secondary Ab alone. Cells were washed and subsequently fixed with PBS containing 1% formaldehyde. Fluorescence intensity was measured using a Coulter Profile flow cytometer (Coulter Electronics, Hialeah, Fla., USA) with logarithmic amplification (3 log scale).

Proliferation assay

T cells were cultured at 10^5 cells per well in 96-well, flat bottomed microtiter plates (NUNC, Roskilde, Denmark) for 5 days, together with 10^4 γ-irradiated (200 Gy) stimulator cells. The CD28 mAb (Dr V. v. Fliethner, Ludwig Institute, Epalinges, Switzerland) was of the IgM isotype and comitogenic in soluble form without crosslinking; it was used as culture supernatant at a final concentration of 25%. The CD5 mAb (9H8, our laboratory) was of IgG3 isotype and comitogenic in soluble form without crosslinking; it was used at a final concentration of 50 ng/ml; 37 kBq [^{3}H]-thymidine (74.0 GBq/mmol, New England Nuclear, Boston, Mass., USA) per well were added for further 18 h of incubation. Thymidine uptake was determined by liquid scintillation counting and expressed as mean cpm of triplicate wells.

Cytokines

Cytokines were titrated and the most effective concentrations were used. Tumor cells were pretreated when indicated by incubating for 24 h (which was shown as optimal) with TNF-α (25 ng/ml, Boehringer-Mannheim) and IFN-γ (250 U/ml, Boehringer-Mannheim). IL-2 (natural human IL-2 provided by Biotest, Dreiech, Germany) was added directly into T cell tumor cell cultures at indicated concentrations.

Propagation of T cells

A total of 1–2×10^6 autologous T lymphocytes were cocultured in 24-well plates with 1–2×10^5 γ-irradiated (200 Gy) stimulator cells and restimulated in weekly intervals under identical conditions. CD28 and CD5 mAbs were added in concentrations, as described. From the third day on of the second stimulation (day 10), replaced medium was supplemented with IL-2 (50 U/ml).

Cytotoxic T cell assay

Cell-mediated cytotoxicity was performed in a standard chromium release assay. Stimulated allogeneic or autologous T cells were incubated for 3 h with ^{51}Cr-labeled tumor cells in the presence of different target cells.

Specific cytotoxicity was calculated according to:

$$\frac{(\text{experimental cpm} - \text{spontaneous cpm})}{(\text{maximal cpm} - \text{spontaneous cpm})} \times 100 \quad (1)$$

Antibody inhibition experiments were performed, including the following mAbs: anti-MHC class I (W6/32, 10 μg/ml, ATCC) and anti-MHC class II (AIMHCII, ascites 1:100).

Results

The impaired immunostimulatory capacity of RCC cell lines is augmented by IFN-γ and TNF-α

We investigated established cell lines from human RCC ($n = 5$) for their ability to induce in vitro a primary allogeneic T lymphocyte response. To this end human resting T lymphocytes from healthy donors (exp.1 + 2) were incubated with irradiated tumor cells and T cell proliferation was analyzed. Table 1 shows representative results obtained with two different donors, demonstrating that no, or only weak, T cell proliferation was induced. In contrast, T cell activation was promoted by the allogeneic B lymphoblastoid cell line (B-LCL) M7 under identical conditions. We did not observe a decrease in T cell responses towards phytohemagglutinin (PHA) in the presence of tumor cells, except with KTCTL-30 (Table 1). Reasons for the immunosuppressive activity of KTCTL-30 were not further addressed.

Phenotypic analysis of RCC cells by immunofluorescence revealed that all five cell lines expressed MHC class I antigens (Fig. 1; not shown, KTCTL-30, -104). In contrast, MHC class II molecules were not expressed constitutively on RCC cells. CD54 was expressed only by KTCTL-195 and -104, whereas only low levels of CD58 were detectable on the cell surface of all RCC cell lines. RCC cells were then incubated with recombinant IFN-γ and TNF-α, cytokines known to enhance several surface molecules (e.g., MHC and CD54). As shown in Fig. 1, such treatment for 24 h resulted in increased expression of MHC class I, CD54, and – to a lesser extent – CD58 on KTCTL-140, -187, and -195. The combination of TNF-α and IFN-γ resulted in an even stronger effect. Remarkable is the induction of MHC class II expression on KTCTL-140, -187, and -195 by IFN-γ and TNF-α, respectively, which was again higher following incubation with both cytokines.

Given the importance of the costimulatory molecules for the induction of T lymphocyte responses, we tested RCC cell lines for their expression of CD80 and CD86 by immunofluorescence. Although not shown here, the cell lines under investigation in this study did not express CD80 or CD86 following treatment with IFN-γ and TNF-α, either alone or in combination.

To analyze how cytokine treatment of RCC impacted on T cell responses, we tested RCC cells that were pretreated with a combination of TNF-α and IFN-γ for their ability to induce T cell proliferation. Table 1 shows that cytokine pretreatment had no effect on T cell activation by KTCTL-30 and KTCTL-104. In contrast, KTCTL-140, -187, and -195 stimulated a low but significant response of resting T lymphocytes after preincubation with both cytokines.

Augmentation of T cell responses towards RCC cell lines by CD5 and CD28 mAbs

Given the rather low effects of cytokine pretreatment on the immunogenicity of RCC cell lines, we next included CD5 and CD28 mAbs in our experiments in order to provide costimulatory signals for the activation of T cells. Figure 2 shows that the addition of a CD28 mAb resulted in a significant response of T lymphocytes towards KTCTL-187 and KTCTL-195, respectively. This response was up to eightfold higher when cytokine-pretreated (IFN-γ + TNF-α) tumor cells were used for stimulation. The CD5 mAb augmented T cell proliferation only towards cytokine-pretreated RCC cells. The strongest T cell responses were observed following stimulation with RCC cells in the presence of CD5 plus CD28 mAbs. However, preincubation with cytokines showed no significant improvement. All other RCC cell lines gave comparable results. It should be noted that the CD5 and CD28 mAbs, either alone or in combination, did not stimulate T cells in absence of RCC.

Table 1 Analysis of T cell proliferation in allogeneic mixed lymphocyte tumor cell culture (MLTC) by (3H) thymidine incorporation. In case of cytokine (*Cytok.*) treatment tumor cells (*KTCTL*) were incubated for 24 h with a combination of tumor necrosis factor -α (25 ng/ml) and interferon-γ (250 U/ml). Note that T cells from two different donors were used in experiments 1 and 2. *med* medium control, *nd* not done, *PHA* phytohemagglutinin

	Cytokine	PHA	med	M7	KTCTL				
					-30	-104	-140	-187	-195
Exp. 1	–	–	177	6.555	282	278	315	193	383
	–	+	54.613	nd	27.962	57.392	65.564	41.540	54.039
Exp. 2	–	–	150	10.250	544	418	756	382	288
	+	–	–	nd	436	588	3.440	1.026	2.226

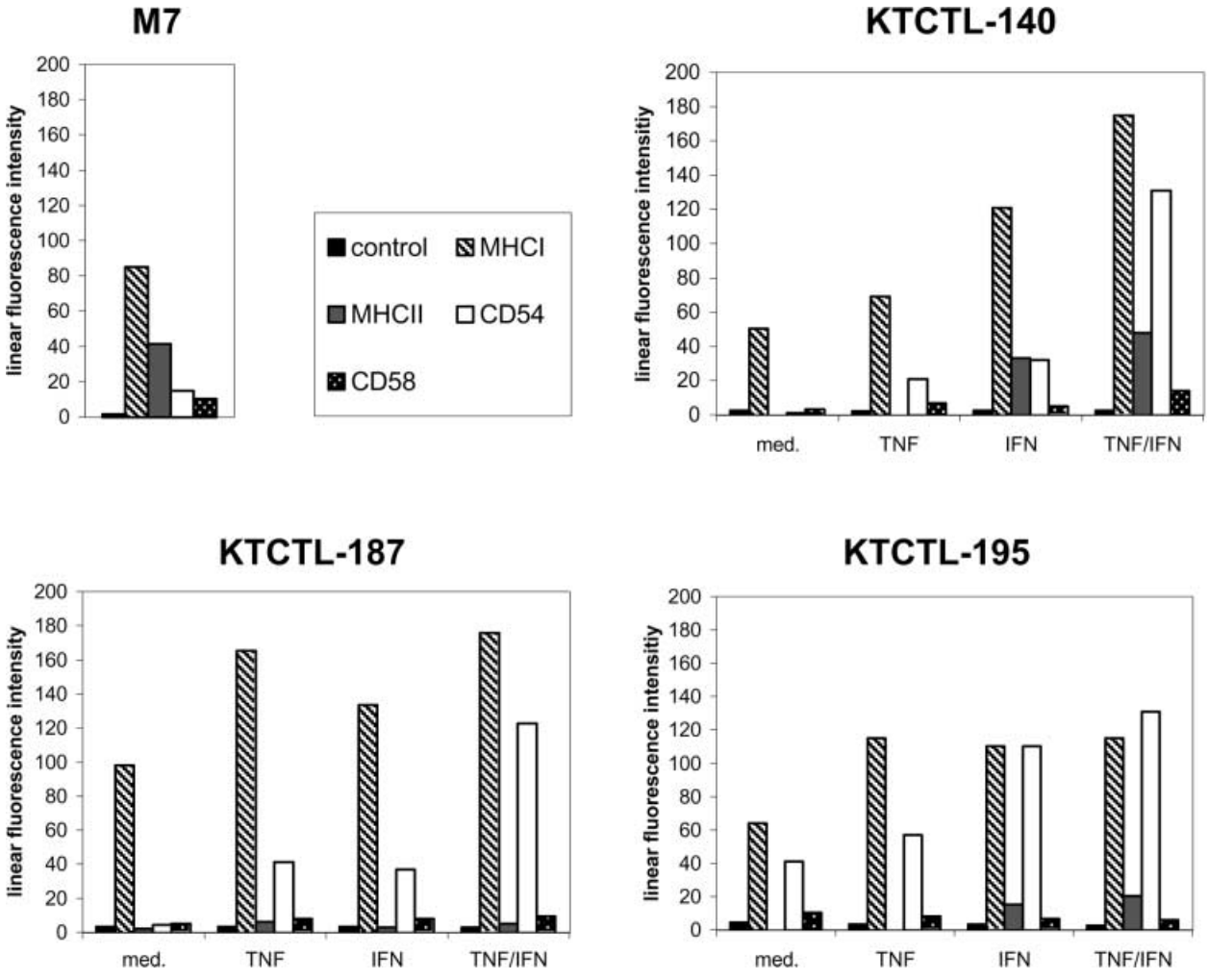

Fig. 1 Immunfluorescence analysis of renal cell carcinoma (RCC) cell lines. RCC cell lines (KTCTL-140, -187, -195) were analyzed for expression of MHC class I (*striped bars*), MHC class II (*gray bars*), CD54 (*open bars*), and CD58 (*dotted bars*) following incubation in medium (*med*), 250 U/ml IFN-γ (*IFN*), 25 ng/ml TNF-α (*TNF*), and a combination of IFN-γ plus TNF-α (*TNF/IFN*), respectively. M7 is a B-lymphoblastoid cell line. Medium-control (*full bars*)

Generation of cytolytic T lymphocytes reactive against an allogeneic RCC cell line

In order to test the ability of RCC cells to promote the generation of cytolytic effector cells, we stimulated allogeneic T cells with KTCTL-187 cells under various conditions. Tumor cells alone, or CD5 plus CD28 mAb alone, did not promote T cell expansion but resulted in a steady decline in the number of T cells. Only stimulation of T cells with tumor cells in the presence of CD5 plus CD28 mAbs allowed T cell expansion up to 50 days in culture. The ratio of CD4+/CD8+ T cells remained constant during this period (approximately 75% CD4+ and 25% CD8+, not shown).

As shown in Fig. 3a, T cells stimulated with cytokine-pretreated KTCTL-187 in the presence of CD5 and CD28 mAbs exhibited a substantial cytolytic activity towards untreated KTCT-187 cells. This activity was only partially inhibited by competing for NK activity and, moreover, cytolysis of K562 cells was low. This suggests that MHC-restricted cytolytic T cells were generated. In contrast, stimulation of T cells by CD5 plus CD28 mAbs in the presence of KTCTL-187 cells that were not pretreated with cytokines, resulted in the generation of cytotoxic T cells with non-MHC restricted activity that were only weakly cytolytic towards KTCTL-187 RCC cells (Fig. 3b).

Generation of cytolytic tumor-reactive T cells following stimulation with autologous RCC cells

We employed the above strategy to stimulate T lymphocytes from an RCC patient (KTCTL-195) against his own tumor cells. The phenotypic characterization of PBMC from this patient revealed 56% CD3+, 58% CD4+, 7% CD8+, and 1% CD56+ cells. This unusually high proportion of CD4+ T cells, which was observed at independent time points, could not be explained by the patient's case history and remains unclear. As observed following stimulation with allogeneic RCC cells, T cell expansion did not occur following

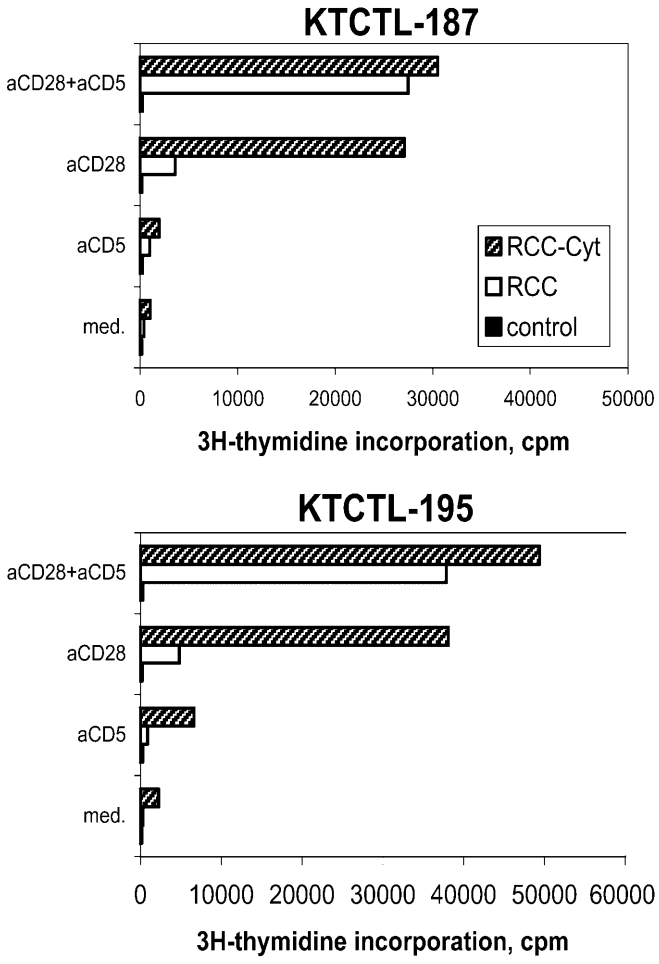

Fig. 2 T cell proliferative response towards renal cell carcinoma (RCC) cells following costimulation with anti-CD28 and anti-CD5 monoclonal antibodies (mAbs). 10^5 T cells were incubated with medium, 10^4 untreated (*open bars*) or IFN-γ plus TNF-α preincubated (*striped bars*) tumor cells. CD28 and CD5 mAb were added in solution. T cell proliferation was determined by thymidine incorporation on day 5

stimulation with cytokine pretreated tumor cells alone or mAbs alone. However, T cells were expanded following stimulation with tumor cells in the presence of mAb (either CD28 or CD5/CD28) and gave rise to T cells (>95% CD3+) of predominantly CD4+ phenotype (85% CD4+, 10% CD8+), as judged by immunofluorescence in both cases (data not shown).

Whereas resting PBMC could not be activated after 2 weeks of stimulation without cytokines, specific lysis of KTCTL-195 was slightly enhanced. Blocking experiments showed only weak inhibition by MHC class I or class II (Fig. 4a).

As shown in Fig. 4b, autologous T cells exhibited cytolytic activity after cytokine pretreatment of tumor cells, whereas their NK activity (lysis of K562 cells) was low. Blocking experiments revealed that a MHC class II specific mAb (12G6) inhibited cytolysis of autologous tumor cells by >70%. In contrast, an mAb directed at MHC class I (W6/32) inhibited only marginally. Fig. 4c shows that the allogeneic tumor cell lines KTCTL-187 (RCC) and SkMel63 (melanoma) were lysed at low efficiency, whereas KTCTL-30 (RCC) was not. Importantly, a strong cytolytic activity was also observed when autologous B lymphoblastoid cells were used as targets. This demonstrates that cytokine pretreated KTCTL-195 induced an MHC class II-restricted cytolytic response that is not restricted to autologous RCC cells.

Discussion

In this study we investigated the use of mAbs directed at costimulatory receptors in order to generate T cell responses against RCC cell lines in vitro. Employing mitogenic mAbs directed against lymphocyte receptors with costimulatory function circumvents the necessity to genetically modify tumor cells to express costimulatory ligands. This is of particular relevance for clinical

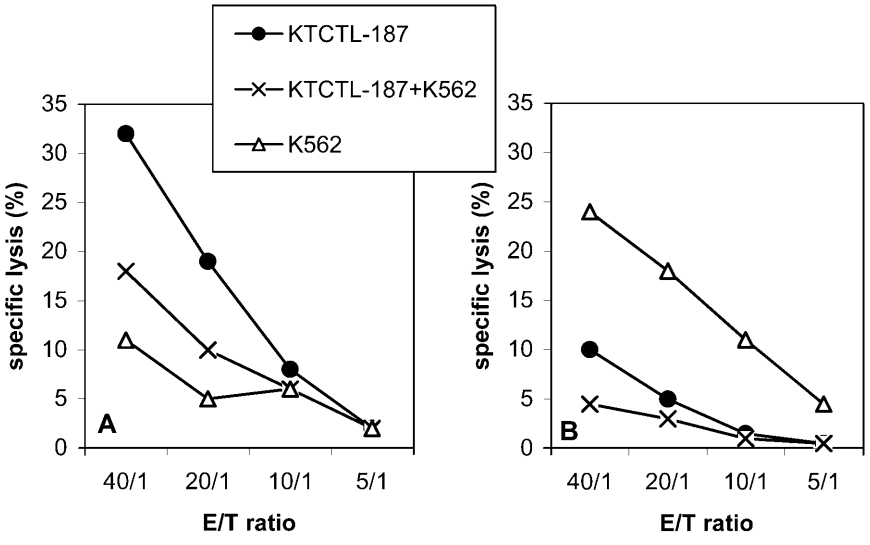

Fig. 3a, b Cytolytic activity of T cells generated following stimulation with the allogeneic renal cell caracinoma (RCC) cell line KTCTL-187. **a** T cells were stimulated with cytokine-pretreated KTCTL-187 (*solid circles*) in the presence of CD5 and CD28 mAbs. This activity was only partially inhibited by competing for natural killer activity (*crosses*). Cytolysis of K562 cells (*triangles*) was low. **b** T cells were stimulated by CD5 and CD28 mAbs in the presence of KTCTL-187 without pretreatment with cytokines. Cytotoxicity of activated T cells were measured after 2 weeks of stimulation by chromium release assay

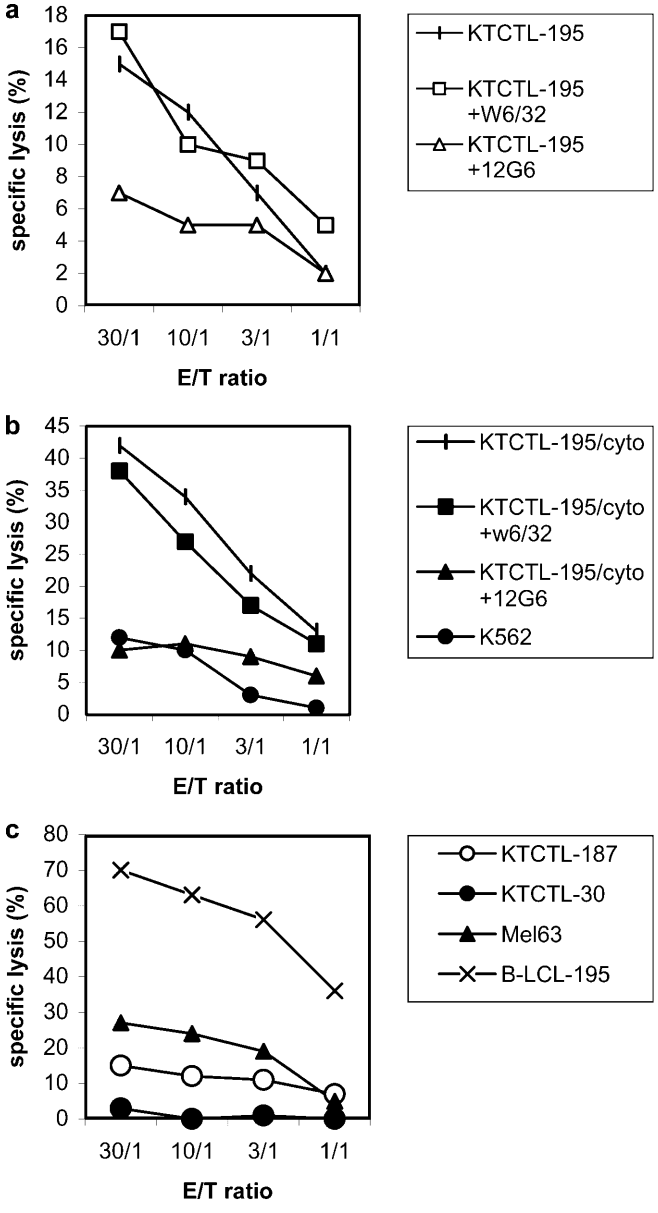

Fig. 4a–c Cytolytic activity of a T cell line generated following stimulation with autologous renal cell carcinoma (RCC) cells (KTCTL-195). T cells were propagated by stimulation with cytokine pretreated autologous RCC cells in the presence of CD28 and CD5 monoclonal antibody (mAb). Cytolytic activity was analyzed on day 14 against different target cells: **a** autologous tumor KTCTL-195 (*vertical lines*), autologous tumor KTCTL-195 plus MHC class I specific mAb W6/32 (*open squares*), autologous tumor KTCTL-195 plus MHC class II specific mAb 12G6 (*open triangles*); **b** autologous cytokine pretreated tumor cells KTCTL-195 in the absence of blocking mAb (*vertical lines*), or in the presence of a MHC class I W6/32 (*solid squares*) and MHC class II 12G6 (*solid triangles*) specific mAb, respectively, and against K562 (*solid circles*); **c**) autologous B lymphoblasts B-LCL-195 (*crosses*) and allogeneic cell lines KTCTL 30 (*solid circle*), KTCTL-187 (*open circle*), SkMel63 (*solid triangles*)

applications since it avoids several problems associated with the in vivo or in vitro use of viral vectors, including time-consuming transfection methods, low transfection efficiencies, low or absent target cell specificity, and risk of uncontrolled viral replication in immune compromised tumor patients.

Besides anti-CD28, several other mAbs, in addition to CD28, have been demonstrated to augment T cell activation induced by anti-CD3, mitogens, or alloantigens. We tested CD28, CD5, CD27, and CD29/49d (VLA-4) mAbs, among which CD28 and CD5 mAbs were found to enhance T cell activation by allogeneic human tumor cells (unpublished observation). Synergistic effects of CD28 and CD5 mAbs, as demonstrated here, were observed previously [27] following anti-CD3 stimulation of human T lymphocytes. In addition, T lymphocytes were induced to proliferate by a combination of anti-CD28 and anti-CD5 in the absence of additional TCR stimulation [46]. Under the experimental conditions employed here, however, the function of CD28 and CD5 mAbs was strictly dependent upon the presence of tumor cells serving as an antigenic stimulus.

Several studies have demonstrated a role for CD5 in delivering accessory signals to human T lymphocytes during activation and proliferation [40, 43]. The fact that anti-CD5 alone did not show any costimulatory effect in the present study may be interpreted to be the result of a weak primary stimulation via the T cell receptor by renal carcinoma cells in comparison to other strong stimuli such as immobilized anti-CD3. (Several studies suggest that the TCR-CD3 complex and CD5 are functionally linked [6, 9].) However, the activity of CD5 triggering became obvious when a CD28-mediated signal, in addition an antigen/MHC signal, had already increased the T cell activation status. Thus, the contribution of signals from both costimulatory pathways CD28 and CD5 yielded a stronger response than CD28 triggering alone.

The influence of IFN-γ and TNF-α, respectively, on the expression of MHC molecules, as well as accessory molecules like CD54, is known and was demonstrated for many cell types, including RCC cells [16, 36, 45]. Such enhanced expression may contribute to improved cell/cell contact and antigen recognition that became most effective in T cell activation only in the presence of costimulatory signals induced by CD28 or CD5/CD28 triggering. In addition, the induction of MHC class II expression facilitated the activation of CD4+ killer T cells that, in turn, may support the induction of CD8+ T lymphocytes.

The results presented here demonstrate that the costimulatory mAbs CD5 and CD28 can be used for the generation and expansion of T cell lines directed against RCC cell lines. Preincubation of RCC cells with IFN-γ/TNF-α improved the generation of MHC-restricted cytolytic T cells. Therefore, a combination of costimulatory signals, i.e., by means of mAbs and increased MHC expression induced by cytokines, seems to be a reasonable approach to generate T lymphocytes directed against tumor antigens in association with MHC molecules. When this approach was tested directly for its efficiency in an autologous RCC model, it was observed, however, that a high proportion of T cells with a specificity unrelated to the RCC cells was induced. These

T cells were most likely directed against autologous MHC class II molecules, since they were inhibited by an MHC class II-specific mAb and were also reactive with autologous B lymphoblasts.

Several reports demonstrated that the expression of CD80 on tumor cells resulted in the generation of CD8+ MHC class I-restricted T cells [20, 44, 21]. The generation of MHC class II-specific T cells, as observed here, may be due to the fact that (1) the induction of MHC class II molecules by IFN-γ/TNF-α or, (2) the combination of CD5 and CD28 mAbs, favored the expansion of MHC class II-specific T cells. However, the fact that the high proportion of CD4+ T cells in the patient was responsible for the predominant generation of MHC class II reactivity cannot be excluded.

It is known that T lymphocytes directed against autologous MHC class II determinants are activated in an autologous mixed lymphocyte reaction (AMLR) following stimulation with B cells or monocytes. Such autoreactive T cells were discussed as representing components of a network of existing but regulated – and thus nonactive – T cells [8]. The increase of costimulatory signals together with MHC class II molecules on RCC cells may have resulted in the activation of autoreactive T cells, as observed in an AMLR.

Our findings and interpretations have the following consequences regarding the use of tumor cells with improved immunogenicity: T cell preparations can be depleted in vitro of autoreactive T cells by focussing particularly on CD8+T cells as responders. This is particularly important because the majority of defined tumor antigens were found to be MHC class I-restricted and recognized by CD8+T cells. With regard to using immunogenic tumor cells as vaccines, the employed methods for improving the potency of a tumor cell to activate T cells should be adjusted so that potential autoreactive T cells will not be activated. Although the stimulation of CD4 helper cells is being discussed as having positive effects on the generation of antitumor responses [42], our findings indicate that "unphysiologic" levels of MHC class II and strong costimulation should be avoided, particularly in combination with autologous tumor cells. However, in cases where allogeneic tumor cells are being employed as vaccines, allogeneic class II molecules may support the activation of tumor-specific T cells by activation of allospecific helper T cells.

Perhaps new methods, including the use of bispecific Abs (antitumor/anticostimulatory receptor), or the chemical coupling of a costimulatory Ab to a tumor cell, could be helpful to find optimal costimulatory levels for enhancing the immunostimulatory capacity of tumor cells, particularly ex vivo [26, 35, 47].

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References

1. Belldegrun A, Muul LM, Rosenberg SA (1988) Interleukin 2 expanded tumor-infiltrating lymphocytes in human renal cell cancer: isolation, characterization, and antitumor activity. Cancer Res 48: 206
2. Blankenstein T, Qin Z, Überla K, Müller W, Rosen H, Volk HD, Diamantstein T (1991) Tumor suppression after tumor cell-targeted tumor necrosis factor α gene transfer. J Exp Med 173: 1047
3. Boon T, Coulie PG, Van den Eynde B (1997) Tumor antigens recognized by T cells. Immunol Today 18: 267
4. Brändle D, Brasseur F, Weynants P, Boon T, Van den Eynde BJ (1996) A mutated HLA-A2 molecule recognized by autologous cytotoxic T lymphocytes on a human renal cell carcinoma. J Exp Med 183: 2501
5. Brouwenstijn N, Gaugler B, Kruse KM, Van der Spek CW, Mulder A, Osanto S, Van den Eynde BJ, Schrier PI (1996) Renal-cell-carcinoma-specific lysis by cytotoxic T-lymphocyte clones isolated from peripheral blood lymphocytes and tumor infiltrating lymphocytes. Int J Cancer 68: 177
6. Burgess KE, Yamamoto M, Prasad KVS, Rudd CE (1992) CD5 acts as a tyrosine kinase substrate within a receptor complex comprising T-cell receptor ζ chain/CD3 and protein-tyrosinase kinases $p56^{lck}$ and $p59^{fyn}$. Proc Natl Acad Sci USA 89: 9311
7. Chen L, Ashe S, Brady WA, Hellström I, Hellström KE, Ledbetter JA, McGowan P, Linsley PS (1992) Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte molecules CD28 and CTLA-4. Cell 71: 1093
8. Clark EA, Ledbetter JA (1994) How B and T cells talk to each other. Nature 367: 425
9. Davies AA, Ley SC, Crumpton MJ (1992) CD5 is phosphorylated on tyrosine after stimulation of the T cell antigen receptor complex. Proc Natl Acad Sci USA 89: 6368
10. Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, Jackson V, Hamada H, Pardoll D, Mulligan RC (1993) Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting antitumor immunity. Proc Natl Acad Sci USA 90: 3539
11. Fearon ER, Pardoll DM, Itaya T, Golumbeck P, Levitsky HI, Simons JW, Karasuyama H, Vogelstein B, Frost P (1990) Interleukin-2 production by tumor cells bypasses T helper function in the generation of an antitumor response. Cell 60: 397
12. Finke JH, Rayman P, Edinger M, Tubbs RR, Stanley J, Klein E, Bukowski R (1992) Characterization of a human renal cell carcinoma specific cytotoxic CD8+ T cell line. J Immunother 11: 1
13. Flad T, Spengler B, Kalbacher H, Brossart P, Baier D, Kaufmann R, Bold P, Metzger S, Bluggel M, Meyer HE, Kurz B, Muller CA (1998) Direct identification of major histocompatibility complex class I-bound tumor-associated peptide antigens of a renal carcinoma cell line by a novel mass spectrometric method. Cancer Res 58: 5803
14. Gansbacher B, Bannerji R, Daniels B, Zier K, Cronin K, Gilboa E (1990) Retroviral vector-mediated γ-interferon gene transfer into tumor cells generates potent and long-lasting antitumor immunity. Cancer Res 50: 7820
15. Gastl G, Finstad CL, Guarini A, Bosl G, Gilboa E, Bander NH, Gansbacher B (1992) Retroviral vector-mediated lymphokine gene transfer into human renal cancer cells. Cancer Res 52: 6229
16. Gastl G, Ebert T, Finstad CL, Sheinfeld J, Gomahr A, Aulitzky W, Bander NH (1996) Major histocompatibility complex class I and class II expression in renal cell carcinoma and modulation by interferon gamma. J Urol 155: 361
17. Gaudin C, Dietrich PY, Robache S, Guillard M, Escudier B, Lacombe MJ, Kumar A, Triebel F, Caignard A (1995) In vivo local expansion of clonal T cell subpopulations in renal cell carcinoma. Cancer Res 55: 685

18. Gaugler B, Brouwenstijn N, Vantomme V, Szikora JP, Van der Spek CW, Patard JJ, Boon T, Schrier PI, Van den Eynde BJ (1996) A new gene coding for an antigene recognized by autologous cytolytic T lymphocytes on a human renal carcinoma. Immunogenetics 44: 323
19. Gimmi CD, Freeman GJ, Gribben JG, Gray G, Nadler LM (1993) Human T cell clonal anergy is induced by antigen presentation in the absence of B7 costimulation. Proc Natl Acad Sci USA 90: 6586
20. Gückel B, Lindauer M, Rudy W, Habicht A, Siebels M, Kaul S, Bastert G, Meuer SC, Moebius U (1995) CD-80 transfected human breast and ovarian tumor cell lines: improved immunogenicity and induction of cytolytic CD8+ T lymphocytes. Cytokines Molec Ther 1: 211
21. Harding FA, Allison JP (1993) CD28-B7 interactions allow the induction of CD8+ cytotoxic T lymphocytes in the absence of exogenous help. J Exp Med 177: 1791
22. Itoh K, Platsoucas CD, Balch CM (1988) Autologous tumor-specific cytotoxic T lymphocytes in the infiltrate of human metastatic melanomas. J Exp Med 168: 1419
23. Jantzer P, Schendel DJ (1998) Human renal cell carcinoma antigen-specific CTLs: antigen-driven selection and long-term persistence in vivo. Cancer Res 58: 3078
24. Kim TY, von Eschenbach AC, Filaccio MD, Hayakawa K, Parkinson DR, Balch CM, Itoh K (1990) Clonal analysis of lymphocytes from tumor, peripheral blood, and nontumorous kidney in primary renal cell carcinoma. Cancer Res 50: 5263
25. Koo AS, Tso CL, Shimabukuro T, Peyret C, deKernion J, Belldegrun A (1991) Autologous tumor-specific cytotoxicity of tumor-infiltrating lymphocytes derived from human renal cell carcinoma. J Immunother 10: 347
26. Kroesen BJ, Bakker A, van Lier RAW, The HAT, de Leij L (1995) Bispecific antibody-mediated target cell-specific costimulation of resting T cells via CD5 and CD28. Cancer Res 55: 4409
27. Ledbetter JA, Martin PJ, Spooner CE, Wofsky D, Tsu TT, Beatty PG, Gladstone P (1985) Antibodies to Tp67 and Tp44 augment and sustain proliferative response of activated T cells. J Immunol 135: 2331
28. Linsley PS (1995) Distinct roles for CD28 and cytotoxic T lymphocyte-associated molecule-4 receptors during T cell activation? J Exp Med 182: 289
29. Luboldt HJ, Kubens BS, Rübben H, Grosse-Wilde H (1996) Selective loss of human leukocyte antigen class I allele expression in advanced renal cell carcinoma. Cancer Res 56: 826
30. Meuer SC, Hussey RE, Fabbi M, Fox D, Acuto O, Fitzgerald KA, Hodgdon IL, Protentis JP, Schlossman SF, Reinherz EL (1984) An alternative pathway of T cell activation: functional role for the 50 KD T 11 sheep erythrocyte receptor protein. Cell 36: 897
31. Mueller DL, Jenkins MK, Schwartz RH (1989) Clonal expansion versus functional clonal inactivation; a costimulatory signalling pathway determines the outcome of T cell antigen receptor occupancy. Ann Rev Immunol 7: 455
32. Neumann E, Engelsberg A, Decker J, Störkel S, Jaeger E, Huber C, Seliger B (1998) Heterogeneous expression of the tumor-associated antigens RAGE-1, PRAME, and Glykoprotein 75 in human renal cell carcinoma: Candidates for T cell based immunotherapies? Cancer Research 58: 4090
33. Oettgen HF (1991) Cytokines in clinical cancer therapy. Curr Opin Immunol 3: 699
34. Pantel K, Schlimok G, Kutter D, Schaller G, Genz T, Wiebecke B, Backmann R, Funke I, Riethmüller G (1991) Frequent downregulation of major histocompatibility class I antigen expression on individual micrometastatic carcinoma cells. Cancer Res 51: 4712
35. Rode HJ, Moebius U, Little M (1999) T cell activation by monoclonal antibodies bound to tumor cells by a cell surface displayed single-chain antibody. J Immunol Meth 224: 151
36. Scheibenbogen C, Keilholz U, Meuer SC, Dengler T, Tilgen W, Hunstein W (1993) Differential expression and release of LFA-3 and ICAM-1 in human melanoma cell lines. Int J Cancer 54: 494
37. Schendel DJ, Gansbacher B, Oberneder R, Kriegmair M, Hofstetter A, Riethmüller G, Segurado OG (1993) Tumor-specific lysis of human renal cell carcinomas by tumor-infiltrating lymphocytes. HLA-A2-restricted recognition of autologous and allogeneic tumor lines. J Immunol 151: 4209
38. Schwartz RH (1990) A cell culture model for T lymphocyte clonal anergy. Science 248: 1349
39. Smith MEF, Marsh SGE, Bodmer JG, Gelsthorpe K (1989) Loss of HLA-A, B, C allele products and lymphocyte function-associated antigen 3 in colorectal neoplasia. Proc Natl Acad Sci USA 86: 5557
40. Spertini F, Stohl W, Ramesh N, Moody C, Geha RSJ (1991) Induction of human T cell proliferation by a monoclonal antibody to CD5. J Immunol 146: 47
41. Tepper RI, Pattengale PK, Leder P (1989) Murine interleukin-4 displays potent antitumor activity in vitro. Cell 57: 503
42. Thomas WD, Hersey P (1998) CD4 T cells kill melanoma cells by mechanisms that are independent of Fas (CD95). Int J Cancer 75: 384
43. Thomas Y, Glickman E, de Martino J, Wang J, Goldstein G, Chess L (1984) Biologic functions of the OKT1 T cell-surface antigen. I. The T1 molecule is involved in helper function. J Immunol 133: 724
44. Townsend SE, Allison JP (1992) Tumor rejection after direct costimulation of CD8+ T cells by B7-transfected melanoma cells. Science 259: 368
45. Vegh Z, Wang P, Vanky F, Klein E (1993) Increased expression of MHC class I molecules on human cells after short-time IFN-γ treatment. Mol Immunol 30: 849
46. Verwilghen J, Vandenberghe P, Wallays G, de Boer M, Anthony N, Panayi GS, Ceuppens JL (1993) Simultaneous ligation of CD5 and CD28 on resting T lymphocytes induces T cell activation in the absence of T cell receptor/CD3 occupancy. J Immunol 150: 835
47. Zeidler R, Reisbach G, Wollenberg B, Lang S, Chaubal S, Schmitt B, Lindhofer H (1999) Simultaneous activation of T cells and accessory cells by a new class of intact bispecific antibody results in efficient tumor-cell killing. J Immunol 163: 1246