T-Cell Activation by Recombinant Receptors: CD28 Costimulation Is Required for Interleukin 2 Secretion and Receptor-mediated T-Cell Proliferation but Does Not Affect Receptor-mediated Target Cell Lysis

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2 The abbreviations used are: mab, monoclonal antibody; TCR, T-cell receptor; CEA, carcinoembryonic antigen; IL, interleukin; ATCC, American Type Culture Collection; CMV, cytomegalovirus; PE, phycoerythrin; PBL, peripheral blood lymphocyte; XTT, 2,3-bis[2-methoxy-4-nitro-5-sulfonyl]-5[phenyl-amino]carbonyl]-2H-tetrazolium; hydroxide; ICAM, intercellular adhesion molecule; MuLV, murine leukemia virus.

ABSTRACT

Recombinant T-cell receptors with antibody-like specificity are successfully used to direct CTLs toward a MHC-independent immune response against target cells. Here we monitored the specific activation of receptor grafted CTLs in the context of CD28 costimulation. Peripheral blood T cells were retrovirally engrafted with recombinant anti-CD30 and anti-carcinoembryonic antigen receptors, respectively, that harbor either the FceRI-γ or the CD3-ξ intracellular signaling domain. Cross-linking of recombinant receptors by solid-phase bound ligand, i.e., CD30 and a carcinoembryonic antigen receptor-specific anti-idiotypic antibody, respectively, induces IFN-γ secretion that is further enhanced by CD28 costimulation of grafted T cells. Induction of interleukin (IL)-2 secretion, in contrast, requires CD28 costimulation in addition to receptor cross-linking, irrespective of T-cell preactivation by anti-CD3 monoclonal antibody plus IL-2 or by anti-CD3 monoclonal antibody plus anti-CD28 monoclonal antibody. Accordingly, induction of IL-2 secretion upon receptor cross-linking by membrane-bound antigen requires CD28/B7 costimulation whereas IFN-γ secretion and cell proliferation does not. The efficiency of cytosis by receptor-grafted CTLs does not depend on and is not affected by CD28 costimulation. The data demonstrate that CTL proliferation, cytokine secretion, and cytosis upon receptor cross-linking are differentially modulated by CD28 costimulation and that cytosis does not require B7 expression on target cells.

INTRODUCTION

T cells grafted with a recombinant receptor combine the advantages of MHC-independent, antibody-based antigen binding with efficient T-cell activation upon specific binding to the receptor ligand (1–3). The antigen binding domain of the receptor consists of a single-chain antibody fragment (scFv) that is derived from a mab.3 The intracellular signaling domain is derived from the cytoplasmic part of a membrane-bound receptor that is capable of inducing cellular activation, e.g., the FceRI receptor γ-chain or the CD3 ξ-chain. T cells engrafted with the recombinant receptor molecule are designed to induce a MHC-independent, antigen-specific immune response upon receptor cross-linking by antigen, thus functioning as a valuable model system for the analysis of receptor-mediated T-cell functions (reviewed in Refs. 4 and 5).

T cells can be permanently grafted with designed, recombinant receptors by retroviral transduction of vector constructs encoding the receptor molecule of choice. Retroviral transduction depends on proliferating cells but is highly inefficient for resting cells. According to the two-signal paradigm, resting T cells can be either preactivated by incubation with an anti-CD3 mab in addition to exogenous IL-2 or by anti-CD28-specific mabs (6) that mimick signaling mediated by receptor molecules. CD28 costimulation, in addition to signaling through the TCR/CD3 complex, is one of the key events required for the complete activation of resting T cells, resulting in cellular proliferation, cytokine secretion, CTL-mediated target cell lysis, and prevention of activation-induced anergy (reviewed in Ref. 7). Studies of CD28 deficient animals suggest that CD28 preferentially serves to amplify and sustain a primary T-cell response (8) and to lower the amount of antigen required to achieve full cellular activation (9). Resting T cells, however, can be alternatively activated via B7-independent pathways or even without any costimulation (10, 11). In contrast to resting T cells, the role of CD28/B7 costimulation in completely activated T cells is much less clear. Once T cells are activated, the triggering of antigen-specific cytolysis via the TCR/ CD3 complex appears to be independent of CD28/B7 costimulation. Moreover, the proliferation of CD8+ T cells seems to be uncoupled from their cytolytic activity but is substantially enhanced by B7 costimulation (12).

In this report, we explored the role of CD28 costimulation for receptor-mediated signaling of T cells grafted with chimeric receptors displaying antibody-like specificity for the CEA and the Hodgkin’s lymphoma-associated CD30 antigen, respectively. The signaling domains of the chimeric receptors are derived from the FceRI γ-chain or the CD3 ξ-chain, respectively. Using this panel of recombinant receptors, we here demonstrate that receptor-mediated target cell lysis is nearly unaffected by CD28/B7 signaling. In contrast, antigen-specific IL-2 secretion and cellular proliferation of grafted T cells is highly dependent on CD28 costimulation and, at least, is partially uncoupled from target cell lysis capacity.

MATERIALS AND METHODS

Cell Lines and Antibodies. LoVo cells (ATCC CCL 229) and LS174T (ATCC CCL 188) cells are CEA-expressing colon carcinoma cell lines. The cell lines were cultured in RPMI 1640 supplemented with 10% (v/v) FCS. The anti-CEA mab BW431/26, the anti-CD30 mab HRS3, the anti-HRS3 idiotypic mab 9G10, and the anti-idiotypic mab BW2064/36 with specificity for the anti-CEA mab were described elsewhere (13–15). The human CD30-Fc fusion protein (16) was purified from supernatants of transfected Chinese hamster ovary cells and myeloma cells, respectively, by affinity chromatography on anthuman IgG antibody agarose (Sigma Chemical Co., Deisenhofen, Germany). The anti-CD3 mab OKT3 was obtained from ATCC (ATCC CRL 8001), and the anti-CD28 mab IES8 was kindly provided by R. van Lier (Department of Immunobiology, University of Amsterdam, Amsterdam, The Netherlands). Both mabs were protein A purified (Pharmacia-Amersham, Freiburg, Germany) from murine ascites or cell culture supernatants.

Generation of B7 Transfectants. The bicistronic expression plasmid pCBneo contains the coding sequences for the B7-1 molecule and the B7-2 molecule, linked by an internal ribosomal entry site sequence, for simultaneous
expression of both B7-1 and B7-2 under control of the CMV early promoter/ enhancer (17). The colorectal carcinoma cell lines LoVo and LS174T, respectively, were transfected with pCB/neo DNA construct using the FuGENE transfection reagent (Roche Diagnostics, Mannheim, Germany), according to the manufacturer’s instructions. After culture for 2 days, transfected cells were selected in the presence of G418 (2 mg/ml; Sigma) and subsequently sub- cloned by limiting dilution. Simultaneous expression of B7-1 and B7-2 on the surface of transfected cells was determined by flow cytometry analysis as described below.

Generation of Chimeric Receptors and Transduction of Peripheral Blood T Cells. Both the generation and expression of the CEA-specific BW431/26-scFv-Fc-γ and -ζ receptors and the CD30-specific HRS3-scFv-γ receptor were described recently in detail (18–20). To express the recombinant receptors in peripheral blood T cells, their expression cassettes were inserted into the retroviral vector pSTITCH (21). Briefly, the DNA sequences coding for the recombinant receptors were flanked with Ncol (5’) and BglII (3’) restriction sites, respectively, by PCR techniques using the following primer oligonucleotides: 5’-TGA TCC AGC TAG TAG TCA ATG TTT CAG GTG CAG ATT TTC-3’ (sense); 5’-GGC AGA TCT GTC GAC CTG TTA GGC AGG GGG CAG-3’ (antisense); 5’-GGC AGA TCT GAG TCG TCG ACT CTA AAG CTA CTG TGG TGG-3’ (γ antisense; restriction sites are underlined). The PCR products were digested and inserted into the Ncol and BamHI sites of the pSP72 vector DNA that also contains parts of the MuLV splice acceptor and long terminal repeat sequences (21). Herewith, the BamHI site was eliminated by using the BamHI/BglII ligation. The resulting DNA was digested with BglII and partially digested with Xhol. Subsequently, DNA fragments comprising the sequences for the recombinant receptor and parts of the MuLV splice acceptor and long terminal repeat sequences were inserted into the BglII and Xhol sites of the E3qB vector DNA constituting the MuLV-derived retroviral expression vectors pSTITCH/BW431/26-scFv-γ and pSTITCH/HRS3-scFv-γ, respectively. To generate GALV-pseudotyped retroviruses for infection of peripheral blood T cells, the retroviral expression vector DNA (6 μg of DNA) was cotransfected into 293T cells by calcium phosphate coprecipitation with the retroviral helper plasmid DNAs pHI70 and pCOLT (each 6 μg of DNA). pHI70 encodes the MuLV gag and pol genes, whereas pCOLT encodes the GALV-envelope gene under control of the CMV promoter/enhancer (21). Peripheral blood lymphocytes from healthy donors were isolated by density centrifugation and cultured for 48 h in RPMI 1640 supplemented with 10% FCS in the presence of IL-2 (400 units/ml; Endogen, Woburn, MA) and OKT3 mab (100 ng/ml). The cells were harvested, washed, resuspended in medium with IL-2 (400 units/ml), and cul- tivated for 48 h with transiently transfected 293T cells. T-Cells were harvested, and receptor expression was monitored by flow cytometric analysis.

Immunofluorescence Analysis. Receptor transfected T cells were identified by two-color immunofluorescence using BW431/26-scFv- and HRS3-scFv-specific anti-idiotypic mabs (both IgG1; 10 μg/ml, respectively), and the anti-CD3 mab OKT3 (IgG2a; 2.5 μg/ml). Bound antibodies were detected by a FITC-conjugated F(ab’)2, antimonuose IgG1 antibody (2 μg/ml) and a PE-conjugated F(ab’)2, antimouse IgG2a antibody (2 μg/ml; both purchased from Southern Biotechnology, Birmingham, AL), respectively. Expression of B7-1 and B7-2 on transfected tumor cells was determined by using FITC-conjugated anti-B7-1 (MAB104) and PE-conjugated anti-B7-2 mabs (HAS.2B7; both purchased from Coulter-Immunotech, Hamburg, Germany), respectively. Expression of CEA was monitored by incubation of tumor cells with an anti-CEA mab (CD3065; Coulter-Immunotech) and subsequently a FITC-conjugated F(ab’)2, antihuman IgG1 antibody (2 μg/ml). Immunofluorescence was ana- lyzed by using a FACSscan cytometer equipped with the CellQuest research software (Becton Dickinson, Mountain View, CA).

Stimulation of Receptor Grafted Peripheral Blood T Cells. Micrometer plates were coated with the anti-CD28 mab 15E8, the anti-CD3 mab OKT3, the anti-BW431/26 idiotypic antibody BW2064/36, or the CD30-Fc fusion protein. Transduced and nontransduced peripheral blood T cells (1 × 10^6 cells/well), respectively, were incubated for 48 h at 37°C in coated micrometer plates. Alternatively, receptor grafted and nontransduced T cells (1.25 × 10^5 to 10 × 10^6/well) were cocultivated for 48 h with B7-1- and B7-2-expressing CEA- and nontransduced CEA- colon carcinoma cells (5 × 10^3/well). The culture supernatants were analyzed for secretion of IFN-γ and IL-2 by ELISA. Briefly, IFN-γ was bound by a solid-phase antihuman IFN-γ mab (1 μg/ml) and detected by a biotinylated anti-human IFN-γ mab (0.5 μg/ml; both purchased from Endogen). IL-2 was bound by a solid-phase antihuman IL-2 antibody (1:250) and detected by a biotinated antihuman IL-2 antibody (1:250; both purchased from Pharmingen). The reaction product was visualized by a combination of a peroxidase-streptavidin conjugate (1: 10,000) and ABTS (both purchased from Roche Diagnostics) as a substrate.

Cell Proliferation of PKH26-labeled T Cells. The membrane of receptor grafted and nontransduced PBLs, respectively, was labeled with the red fluo- rescent dye PKH26 (Sigma) as described recently (22, 23). PKH26-labeled, receptor grafted and nontransduced PBLs, respectively, were cocultured for 72 h with B7-expressing or nontransfected CEA- colon carcinoma cells (5 × 10^3 cells/well), respectively. Nonadherent PBLs were harvested and analyzed by flow cytometry. The analysis was performed in triplicate, and the cells from individual wells were pooled before analysis. The lymphocyte population was defined setting forward and side-scatter parameters. Dead cells were excluded from analysis by staining with propidium iodide. Cell division was accompanied by reduced intensity of the membrane dye PKH26. Cell division was monitored by PKH26 fluorescence intensity; histogram markers were set with >97.5% of freshly labeled viable lymphocytes laying inside the defined histogram region.

XTT-based Cytotoxicity Assay. Specific cytotoxicity of receptor grafted T cells to target cells was monitored by XTT-based colorimetric assay according to Jost et al. (24). Briefly, receptor grafted and nontransduced T cells (1 × 10^6 cells/well) were cocultivated in triplicate in round-bottomed micro- tier plates with B7-expressing or nontransfected CEA- LoVo and LS174T cells, respectively. After 48 h, supernatants were harvested and in addition analyzed for cytokine secretion. XTT reagent (1 mg/ml; Cell Proliferation Kit II; Roche Diagnostics) was added to the cells and incubated for 90 min at 37°C. Reduction of XTT to formazan by viable tumor cells was monitored colori- metrically at an absorbance wavelength of 450 nm and a reference wavelength of 630 nm. Maximal reduction of XTT was determined as the mean of six wells containing only tumor cells, and the background was determined as the mean of wells containing RPMI 1640 and 10% FCS. The nonspecific formation of formazan attributable to the presence of effector cells was determined from triplicate wells containing effector cells alone but at the same numbers as in the corresponding experimental wells. The number of viable tumor cells [%] was calculated as follows:

\[
\text{viability} = \frac{\text{Absorbance}_{\text{experimental wells}} - \text{Absorbance}_{\text{tumor cells without effectors}}} {\text{Absorbance}_{\text{tumor cells without effectors}} - \text{medium}} \times 100
\]

To demonstrate the specificity of BW431/26-scFv-Fc-γ/ζ-receptor-mediated lysis of CEA- tumor cells, the assay was also performed in the presence of the anti-BW431/26 idiotypic mab BW2064/36 and as a control in the presence of the anti-HRS3 idiotypic mab 9G10 (each 2 μg/ml).

RESULTS

Expression of Recombinant Receptors in Peripheral Blood Lymphocytes. The DNA expression cassettes encoding the recombinant anti-CEA and anti-CD30 T-cell receptors, respectively, were inserted into the retroviral expression vector pSTITCH. Peripheral blood T cells were isolated and transduced as described in “Materials and Methods.” T cells expressing the anti-CEA or anti-CD30 receptor were identified by two-color fluorescence using BW431/26-scFv- and HRS3-scFv-specific anti-idiotypic mabs, respectively, and the anti-CD3 mab OKT3 (Fig. 1).
26-scFv-Fc-γ receptor grafted lymphocytes, respectively, were incubated for 48 h. Analyses of the supernatants revealed that receptor-grafted T cells secrete high amounts of IFN-γ upon specific stimulation with the receptor ligand as well as upon nonspecific stimulation via anti-CD3 or in the presence of anti-CD28 mab alone (Fig. 2A). The amount of secreted IFN-γ upon receptor-mediated stimulation by binding to ligand is furthermore enhanced by CD28 costimulation. Receptor-mediated IL-2 secretion, however, was only observed in the presence of CD28 costimulation. In contrast, no IL-2 secretion was detected upon cross-linking of the recombinant receptor or of CD3 in the absence of CD28 costimulation (Fig. 2B). We conclude that T cells grafted with the anti-CD30 receptor and the anti-CEA receptor, respectively, both require CD28 costimulation in addition to antigen-specific stimulation for the induction of IL-2 secretion.

We asked whether CD28 costimulation of lymphocytes prior to retroviral transduction or the signaling domain of the recombinant receptor modulates IL-2 secretion upon antigen-specific stimulation of receptor-grafted T cells. We used two recombinant receptors (BW431/26-scFv-Fc-γ; BW431/26-scFv-Fc-ζ) that have the same extracellular antigen binding domain but different intracellular signaling domains, either the FcεR1 γ-chain or the CD3 ζ-chain. Peripheral blood T cells were stimulated with the anti-CD3 mab OKT3 plus either IL-2 or the anti-CD28 mab 15E8 prior to engraftment with the recombinant receptor. Antigen-specific receptor cross-linking induced IFN-γ secretion that was furthermore enhanced by CD28 costimulation (Fig. 3, A and B). In contrast, IL-2 secretion was only recorded upon receptor cross-linking in the presence of CD28 costimulation, irrespective of T-cell preactivation by anti-CD3 mab plus IL2 or by anti-CD3 mab plus anti-CD28 mab. Furthermore, T cells grafted either with the recombinant γ- or ζ-chain receptor require both CD28 costimulation for induction of specific IL-2 secretion.

We also tested whether the requirement of CD28 costimulation can be substituted by high concentrations of the receptor ligand. T cells grafted with the BW431/26-scFv-Fc-γ receptor were incubated for 48 h in microtiter plates coated with increasing amounts of either the anti-BW431/26 idiotypic mab BW2064/36 or, for comparison, the anti-CD3 mab OKT3 in the presence/absence of a constant amount of the anti-CD28 mab 15E8. Analysis of the culture supernatants revealed that the immobilized anti-idiotypic mab BW2064/36 as well as the anti-CD3 mab alone efficiently induced IFN-γ secretion (Fig. 4, B and C), whereas no IL-2 secretion was observed even after stimulation with antibodies in coating concentrations of 20 μg/ml (Fig. 4, A and C). CD28 costimulation, on the other hand, resulted in both recombinant receptor- and CD3-mediated secretion of high amounts of IL-2. Moreover, CD28 costimulation lowered the threshold for IFN-γ secretion dramatically (Fig. 4, B and D). Stimulation of receptor-grafted T cells with low concentrations of the anti-CD28 mab without secondary signaling via the recombinant receptor or CD3 induced only low amounts of IFN-γ and no IL-2 secretion (Fig. 4, E and F).
Taken together, the data demonstrate that: (a) both recombinant receptor-mediated signaling and signaling via the endogenous CD3/TCR complex induce IFN-γ secretion but no IL-2 secretion; (b) CD28 costimulation lowers the threshold for IFN-γ secretion; (c) CD28 costimulation is required for both CD3 and recombinant receptor-mediated IL-2 secretion, irrespective of the signaling chain of the receptor (FcεRI-γ or CD3-ζ) and of T-cell preactivation with or without CD28 costimulation; and (d) the requirement of CD28 costimulation cannot be substituted by increasing amounts of the receptor ligand.

IFN-γ and IL-2 Secretion of Receptor-grafted T Cells upon Coculture with B7-expressing Tumor Cells. Because specific induction of IFN-γ and IL-2 secretion differs in terms of their requirements for CD28 costimulation, we asked whether B7 expression on target cells modulates: (a) cytokine secretion of receptor grafted T cells; (b) specific cytolysis of target cells; and (c) T-cell proliferation upon receptor cross-linking. Two colorectal cancer lines (LoVo and LS174T) that express similar amounts of CEA (data not shown) were transfected with the expression vector pCB7neo that contains a cassette for the expression of both B7-1 and B7-2 linked via an internal ribosomal entry site sequence under control of the CMV promoter/enhancer. Isolated cell clones simultaneously express B7-1 and B7-2 (termed as B7 positive), as demonstrated by flow cytometry (data not shown). We cocultivated BW431/26-scFv-Fc-ζ receptor grafted and nontransduced T cells with B7-expressing and nontransfected LoVo and LS174T cells, respectively, and recorded the amount of IFN-γ and IL-2 secreted into the culture supernatant. Cocultivation of receptor-grafted T cells with B7-positive tumor cells resulted in the secretion of substantially higher amounts of IFN-γ compared with cocultivation with B7 negative tumor cells (Figs. 5, B and D). The effect was similar for both B7-expressing LoVo cells and LS174T cells, respectively. Cocultivation of nontransduced T cells with B7-positive or -negative target cells induced no IFN-γ secretion. Cocultivation of receptor-grafted T cells with B7-negative LoVo cells resulted in IL-2 secretion, although in low amounts (Fig. 5A), whereas cocultivation with B7-negative LS174T cells did not (Fig. 5C). In contrast, costimulation of recombinant receptors with membrane-bound B7 resulted in secretion of high amounts of IL-2 (Fig. 5, A and C). Cocultivation of nontransduced lymphocytes with B7-positive or -negative tumor cells, however, did not induce IL-2 secretion. These data demonstrate that B7-mediated signaling alone is not sufficient for cytokine secretion.

Recombinant receptor-mediated low IL-2 secretion in the presence of nontransfected LoVo cells is in contrast to the strict requirement of CD28 costimulation on solid-phase bound receptor ligand. This effect may be attributable to the expression of ICAM-1 on tumor cells, which also provides costimulatory activity (25). Flow cytometric analysis of LoVo and LS174T tumor cells revealed that LoVo cells express only very low amounts of ICAM-1 (data not shown), suggesting that high levels of ICAM-1 delivered the costimulatory effect.
signal to induce low IL-2 secretion upon cocultivation of receptor grafted T cells with B7-negative LoVo cells (Fig. 5A).

**Specific Cytolysis of B7-expressing Tumor Cells by Receptor-grafted T Cells.** We studied the cytolytic activity of T cells grafted with the BW431/26-scFv-Fc-ξ receptor toward B7-expressing tumor cells by applying a tetrazolium salt (XTT)-based cytotoxicity assay. This assay is suitable for monitoring of target cell lysis over an extended period of time, even with low numbers of effector cells (24). Coincubation of BW431/26-scFv-Fc-ξ receptor grafted T cells with CEA+ LoVo and LS174T tumor cells, respectively, resulted in highly efficient lysis of CEA+ target cells, whereas T cells lacking the CEA-specific receptor were poorly cytolytic (Fig. 6, A and B). Co-stimulation via B7, however, only slightly amplified the cytolytic activity of receptor grafted T cells against B7-expressing LoVo cells but not against B7-positive LS174T cells. Target cell lysis is restricted to the recombinant receptor because incubation of grafted T cells in the presence of the anti-BW431/26 idiotypic mab BW2064/36 abolished specific cytolysis of tumor cells, whereas an isotype-matched control mab (9G10) did not (Fig. 6, C and D).

**Proliferation of Receptor-grafted T Cells upon Incubation with B7-expressing Tumor Cells.** To monitor T-cell proliferation upon receptor triggering in the context of B7-CD28 costimulation, we labeled the cell membrane of receptor grafted lymphocytes with the red fluorochrome PKH26. Labeled lymphocytes grafted with BW431/26-scFv-Fc-γ and BW431/26-scFv-Fc-ξ receptors, respectively, were incubated in the presence of B7-positive and -negative LS174T tumor cells, respectively. After 72 h, nonadherent cells were harvested and analyzed by flow cytometry as described in "Materials and Methods." Incubation with LS174T cells induced proliferation of lymphocytes grafted with γ- and ξ-chain receptors, respectively (Fig. 7). Receptor-
triggered proliferation was significantly enhanced by incubation with B7-expressing LS174T cells. As controls, lymphocytes with and without specific receptor did not proliferate significantly in the absence of CEA<sup>1</sup> tumor cells. Proliferation of receptor grafted lymphocytes is specifically mediated by the anti-CEA receptor because T cells without specific receptor are not induced to proliferate upon incubation with CEA<sup>1</sup> tumor cells, irrespective of their B7 expression. These data indicate that CD28 costimulation is not required, but substantially enhances, antigen-specific T-cell proliferation.

**DISCUSSION**

In this report, we monitored specific T-cell activation via recombinant receptors in the context of CD28 costimulation using both solid-phase bound receptor ligands as well as ligand-expressing target cells. Our data demonstrate that receptor-mediated target cell lysis does not require CD28 costimulation; however, receptor-mediated cytokine secretion and T-cell proliferation depend on CD28 costimulation. Specific receptor signaling upon binding to solid-phase ligand without CD28 costimulation induces IFN-γ secretion but no IL-2 secretion, despite T-cell preactivation by either anti-CD3/IL-2 or anti-CD3/CD28. This partial inefficiency in receptor-mediated cellular activation could not be overcome by increasing the amount of receptor ligand. Receptor cross-linking and simultaneous CD28 costimulation is required for the efficient induction of IL-2 secretion.

The data reported here have substantial consequences for the concept of MHC-independent cellular targeting by recombinant receptor molecules:

- **(a)** specific target cell lysis by receptor-grafted T cells is independent of CD28 costimulation, allowing efficient cytolysis of those antigen-expressing tumor cells that lack costimulatory molecules.
- **(b)** IL-2 secretion by receptor-grafted T cells requires CD28 costimulation. Because IL-2 plays a key role for Th1-based cellular immunity (26), targeting of tumor cells lacking costimulatory molecules by receptor-grafted T cells will only result in a limited cellular immune response. The acquisition of additional cellular effectors, e.g. natural killer cells, and the maintenance of a prolonged antitumor cell reactivity are likely to require CD28 costimulation, despite high IFN-γ secretion levels of receptor-grafted T cells. On the other hand, the restricted capacity of receptor-grafted T cells to secrete IL-2 in the absence of CD28 costimulation will limit the immune response, thus preventing autoaggression of activated receptor T cells against normal tissues. From a practical viewpoint, CD28 costimulation may be useful to enhance receptor-mediated cytolysis in the immunotherapy of malignancies. Blocking of CD28 costimulation may be the strategy of choice to limit the immune response.
- **(c)** Cytokine secretion upon cross-linking of the recombinant receptor and of the CD3/TCR complex, respectively, was found to be modulated in a similar fashion by CD28 costimulation. In accordance to our data, target cell lysis and effector cell proliferation upon signaling via the endogenous T-cell receptor in preactivated T cells are differentially modulated by CD28 costimulation (12). We conclude that cellular activation of grafted T cells via the recombinant receptor is regulated and integrated in a similar fashion as T-cell activation via the common CD3/TCR complex. Recombinant T-cell
receptor molecules as applied in this study may therefore represent a valuable tool for the analysis of T-cell activation in peripheral blood lymphocytes.

(d) Target cell lysis via a recombinant TCR was demonstrated recently to be coregulated by the adhesion molecule ICAM-1 (27). Here we demonstrate that CD28 costimulation has a minor effect on the efficiency of target cell lysis but substantially influences other activation parameters, such as cell proliferation and cytokine secretion. On the other hand, target cells with high expression of ICAM-1, as demonstrated for LoVo cells, can induce receptor grafted T cells to secrete IL-2 without CD28 costimulation but to a much lower extent than CD28 costimulation.

Taken together, the data suggest that in the case of LoVo cells, CD28 costimulation is, at least in part, substituted by the ICAM-1 receptor. TCR.

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REFERENCES


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