Cancer Immunol Immunother (1995) 41: 162–168

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ORIGINAL ARTICLE

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Immunotherapy of human colon cancer by antibody-targeted superantigens

Received: 22 May 1995 / Accepted: 5 June 1995

Abstract T lymphocytes generally fail to recognize human colon carcinomas, suggesting that the tumour is beyond reach of immunotherapy. Bacterial superantigens are the most potent known activators of human T lymphocytes and induce T cell cytotoxicity and cytokine production. In order to develop a T-cell-based therapy for colon cancer, the superantigen staphylococcal enterotoxin A (SEA) was given tumour reactivity by genetic fusion with a Fab fragment of the monoclonal antibody C242 reacting with human colon carcinomas. The C242Fab-SEA fusion protein targeted SEA-reactive T cells against MHC-class-II-negative human colon carcinoma cells in vitro at nanomolar concentrations. Treatment of disseminated human colon carcinomas growing in humanized SCID mice resulted in marked inhibition of tumour growth and the apparent cure of the animals. Therapeutic efficiency was dependent on the tumour specificity of the fusion protein and human T cells. Immunohistochemistry demonstrated massive infiltration of human T cells in C242Fab-SEA-treated tumours. The results merit further evaluation of C242Fab-SEA fusion proteins as immunotherapy in patients suffering from colon carcinoma.

Key words Superantigens · SCID mice · Colon carcinoma · Therapy

Introduction

Colon cancer is one of the commonest cancer types among humans. Patients suffering from colon cancer with local lymph node metastases (Duke stage C) or advanced metastatic disease (Duke stage D) have a median survival time of less than 5 years and 1 year respectively. Current available standard chemotherapeutic protocols do not improve survival more than marginally.

Recent progress in the molecular understanding of T cell recognition of target cells has raised the hope of developing immunotherapy against cancer. Colon cancer is, however, considered to be a tumour of low immunogenicity not responding to interleukin-2 (IL-2) and lymphokine-activated killer cell/tumour-infiltrating lymphocyte (LAK/TIL) therapy [16]. Moreover, metastatic colon cancer has been demonstrated to down-regulate MHC class I antigens [10], suggesting that gene therapy using DNA-mediated transfer of costimulatory molecules, e.g. CD80 [2], will have limited impact. In contrast, numerous highly expressed tumour-associated antigens have been defined by mAb [13].

In order to circumvent the need for the immunogenic peptide/MHC complex in eliciting an immune attack on colon cancer cells, we have produced recombinant fusion proteins of colon-cancer-specific mAb and superantigens. This has allowed us to target superantigenicity onto poorly immunogenic colon cancer cells and evoke a therapeutic antitumour immune response. Superantigens are a collection of bacterial and viral proteins, which activate a large fraction of T cells in a MHC-class-II- and TCRVβ-dependent manner [14]. In order to direct the immune response against colon cancer cells, we introduced a novel binding specificity in the superantigen staphylococcal enterotoxin A (SEA) by creating a fusion protein of the colon-cancer-specific mAb C242 [9] and SEA. The C242Fab-SEA fusion protein was shown to suppress strongly the growth of human colon carcinoma in SCID (severe combined immunodeficiency) mice humanized with human peripheral blood mononuclear cells. This effect was antigen-specific and involved targeting of tumour-infiltrating human T lymphocytes.

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Materials and methods

Reagents

RPMI-1640 medium was obtained from Gibco, Middlesex, England. The complete medium had a pH of 7.4 and contained 2 mM L-glutamine (HyClone, Cramlington, Neb.), 0.01 M HEPES (HyClone, Cramlington, Neb.), 1 mM $NaHCO_3$ (Biochrom KG, Germany), 0.1 mg/ml gentamicin sulphate (Kibbutz Beit Haemek, Israel), 1 mM sodium pyruvate (HyClone, Cramlington, Neb.), 0.05 mM 2-mercaptoethanol (Sigma Co., St. Louis, Mo.) and 10% fetal bovine serum (Gibco, Middlesex, England). Human recombinant IL-2 was obtained from Cetus Corp., USA. Mitomycin C was purchased from Sigma Co., USA. Phosphate-buffered saline (PBS) with or without magnesium and calcium was acquired from Imperial, England, and supplemented with or without 1% BALB/c mouse serum. Recombinant SEA was expressed in *E. coli* and purified to homogeneity as earlier described [6]. Murine IgG1 C242 mAb reacting with human colon carcinomas [9] and murine IgG2a C215 mAb [1] reacting with human epithelial cells were obtained from Pharmacia, Stockholm, Sweden. FabC242 fragment was obtained after papain cleavage of the C242 mAb and purified by protein G affinity chromatography [6].

Cloning of C242Fab-SEA

The C242 hybridoma cell line was used for molecular cloning of the cDNA encoding the immunoglobulin heavy chain and κ-chain. Polyadenylated RNA was extracted from 10^7 hybridoma cells, converted to double-stranded cDNA and cloned into the phage-λ-based vector Uni-ZAP (Stratagene Inc., La Jolla, California). The resulting cDNA library of 85 000 independent clones was screened using hybridization probes covering the genes encoding the first constant domain of the heavy chain and the κ constant domain. Positive clones were used to prepare cDNA containing pBluescript SK(–) plasmids. The resulting cDNA-containing plasmids were characterized by restriction enzyme mapping, and plasmids containing an insert of the expected size were sequenced. The cloned C242 heavy chain Fd gene fragment was fused to the SEA gene [6] and expressed as a bicistronic transcription unit together with the C242 light κ chain. The vector pKP941 was used for expression of the fusion protein in *E. coli*. The essential parts included in this vector are the replication functions from pBR322, the kanamycin-resistance gene and a gene encoding C242Fab-SEA under control of an isopropyl-β-D-thiogalactopyranoside(IPTG)-inducible promoter [6] and followed by two copies of phage fd transcription terminators. The vector also includes the *lac* repressor gene (*lacI*), which enables tight control of the promoter. The fusion protein is expressed from a bicistronic unit; the first gene fusion encodes an *E. coli*-derived signal peptide, the Fd part of the heavy chain fused to SEA, and the second gene fusion a synthetic consensus *E. coli* signal peptide followed by the κ chain. The cloning and expression of C215Fab-SEA, used as a control fusion protein in the cytotoxicity assay, have been described [6].

Expression and purification of C242Fab-SEA

E. coli K12 strain UL635 (xylI, *ara14*, $T4^R$, ΔompT) was used as host for the production. Fermentation was performed by a fed-batch procedure [6]. Following consumption of the initial glucose, the glucose feed was controlled by the amount of dissolved oxygen. At an absorbance at 600 nm of about 50 the production of C242Fab-SEA was induced by addition of IPTG to 0.05 mM. The product was purified from microfiltered clarified culture medium by affinity chromatography on protein-G–Sepharose. After washing, C242Fab-SEA was eluted with a buffer of low pH and further purified by ion-exchange chromatography on SP-Sepharose hp (Pharmacia Biotechnology, Uppsala). Finally, buffer exchange on Sephadex G-25 fine (Pharmacia Biotechnology) was used to formulate the protein in phosphate buffer. The final C242Fab-SEA preparation was more than 99% pure as determined by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS-PAGE) analyses and contained less than 5 EU endotoxin/mg protein.

Cells

The human colon carcinoma cell lines Colo205 and WiDr and the B cell lymphoma Raji were obtained from the American Type Culture Collection (Rockville, Md.). The cells were cultured in complete medium and repeatedly tested for *Mycoplasma* contamination with the Gen-Probe *Mycoplasma* T. C. test (Gen-Probe Inc., San Diego, USA). Peripheral blood mononuclear cells (PBM) were obtained from blood donors at the University Hospital of Lund. The cells were isolated by density centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden) as described [4]. SEA-activated T cell lines were produced by activation of PBM [4]. The PBM were stimulated at a concentration of 2×10^6 cells/ml with mitomycin-C-treated SEA-coated B cell lymphoma cells (BSM) in complete medium as described [4]. The T cell lines were restimulated biweekly with 20 U/ml human recombinant IL-2 and weekly with mitomycin-C-treated SEA-coated BSM cells. The T cell lines were more than 95% $CD3^+$, as determined by fluorescence-activated cell sorting analyses.

Iodination of C242Fab-SEA, mAb C242 and SEA

A 20-µg sample of the different proteins was radiolabelled with 1–5 mCi $Na^{125}I$ (NEN, Boston, Mass.) using Enzymobeads with the lactoperoxidase technique as earlier described [6]. The reaction was terminated by quenching with sodium azide and protein-bound radioactivity was separated from free iodine by filtration through a PD-10 column (Pharmacia). Conditions were chosen to obtain a specific activity of 10–40 µCi/µg.

CA242 binding assay

The mAb C50 (CanAg Diagnostics, Gothenburg, Sweden), recognizing an epitope on the CA242 antigen distinct from mAb C242, was coated on MaxiSorp microtitre plates (Nunc, Denmark) at a concentration of 3.75 µg/ml. Blocking was performed with 1% bovine serum albumin in 10 mM phosphate-buffered saline, pH 7.4 with 0.05% Tween-20 (PBS/Tween). The CA242 extract (spent medium from Colo205 cells, 300 arbitrary units/ml; Pharmacia CanAg), 100 µl/well, was allowed to react for 1 h. All incubations were performed at room temperature on a shaker platform with three washings with PBS/Tween between each step. Finally, ^{125}I-labelled intact C242 or C242Fab-SEA, 50 µl/well, was applied in triplicate at appropriate serial dilutions and incubated for 1 h. After washing, the wells were snapped off for measurement of radioactivity in a gamma counter. The apparent dissociation constant, K_d, and the number of binding sites at saturation, N, were calculated according to Scatchard.

MHC class II binding assay

Determination of K_d of C242Fab-SEA and SEA binding to MHC class II^+ Raji cells was performed in a suspension cell binding assay using ^{125}I-labelled proteins, as earlier described [6].

Cytotoxicity assay

The cytotoxicity was measured in a standard 4- to 6-chromium-release assay employing ^{51}Cr-labelled Colo205 or Raji cells as target cells and human T cells as effector cells ar earlier described [4]. The percentage specific cytotoxicity was calculated by using the average of the radioactivity (cpm) in the formula: specific cytotoxicity (%) = [experimental release (cpm) – spontaneous release (cpm)]/[total release (cpm) – spontaneous release (cpm)].

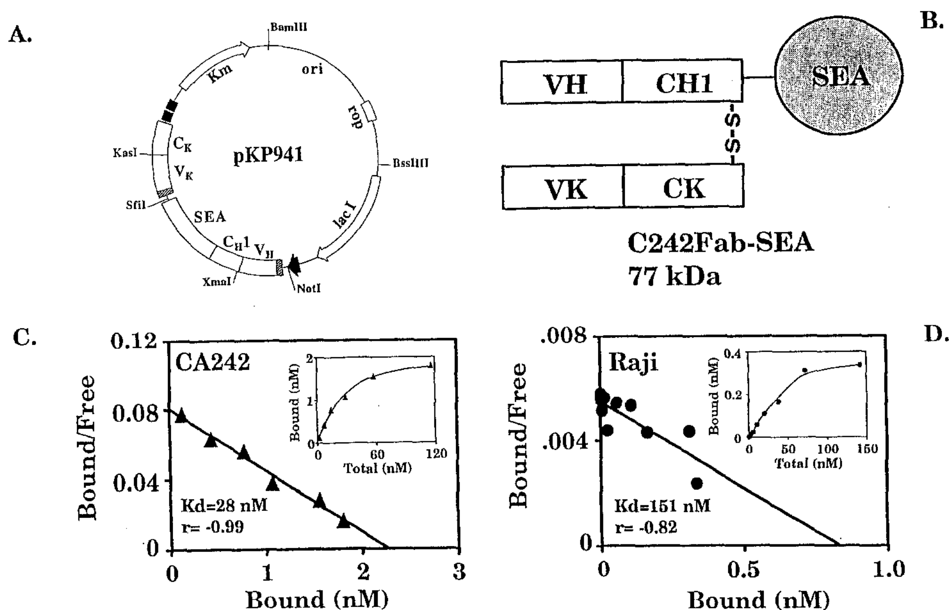

Fig. 1. A The expression plasmid pKP941 encoding the C242Fab-SEA fusion protein is outlined. The C242Fab-SEA fusion protein is expressed from a bicistronic unit; the first gene fusion encodes an *E. coli*-derived signal peptide, the F_d part of the heavy chain fused to SEA, and the second gene fusion a synthetic consensus *E. coli* signal peptide followed by the κ-chain. **B** Schematic structure of the C242Fab-SEA fusion protein. The molecular mass of 77 kDa was determined by sodium dodecyl sulphate/polyacrylamide gel electrophoresis (SDS-PAGE) under non-reducing conditions. **C, D** Scatchard plots and saturation curves (*inserts*) for binding of ^{125}I-C242Fab-SEA fusion protein to immobilized CA242 antigen from Colo205 cells (**C**) and to MHC class II$^+$ Raji cells (**D**). The iodinated fusion protein exhibited intact binding activity as analysed in competition experiments with ^{125}I-C242Fab-SEA diluted with the unlabelled fusion protein at different ratios. Each point in **C** and **D** represents the mean of triplicates

Analysis of cytokine production

Samples of 2×10^6 PBM/ml medium were incubated with varyious amounts of SEA or C242Fab-SEA for the times indicated. The cell-free supernatant was tested for IL-2 activity by using an IL-2 enzyme-linked immunosorbent assay (ELISA; Immunotech International, Marseilles, France), for tumour necrosis factor (TNF) by employing a cytotoxicity bioassay with the TNF-sensitive WEHI 164 clone 13 cells [7] and for interferon γ (IFNγ) content by employing an IFN-ELISA (Medgenix, Brussels, Belgium).

Tumor cell growth inhibition assay

Tests were performed in 96-well, flat-bottomed microtitre plates in complete medium as described [5]. Briefly, 5×10^3 WiDr cells were added to each well, followed by reagents and effector cells in a total volume of 200 µl. Cells were cultured for 6 days at 37 °C. After the culture period, the number of remaining tumour cells was determined using an 3-(4,5-dimethylthiazol-2-µl)-2,5-diphenyltetrazolium bromide (MTT) assay an the A_{540} was measured in an ELISA reader [5]. Data are given as percentage tumour growth inhibition, which was calculated as $100 - [A_{test} - A_b)/(A_C - A_b)] \times 100$, where A_{test} indicates the absorbance of tumour cells grown in the presence of effector cells or culture supernatants, A_b the absorbance of wells containing culture medium only and A_c indicates the absorbance of tumour cells grown in medium alone. Each value represents the average of quadruplicate samples. Standard deviations were routinely less than 10% of the mean.

In vivo tumour therapy in SCID mice

Severe combined immunodeficient (SCID) female mice (C.B-17) 2–3 months old were obtained from Bommice, Ry, Denmark, and kept under pathogen-free conditions. The mice were injected intraperitoneally (i.p.) with Colo205 cells in 0.2 ml PBS/1% BALB/c mouse serum. Unless otherwise specified, the animals were injected i.p. with PBM in 0.2 ml PBS/1% BALB/c mouse serum the following day. At various times after injection of lymphoid cells all animals were injected intravenously (i.v.) with C242Fab-SEA, SEA, C242Fab in 0.2 ml PBS/1% BALB/c mouse serum or vehicle alone. Generally two additional i.v. injections of the test substance were given at 3-day intervals. After 4–6 weeks of growth, the untreated animals had a total tumour weight of 800–2500 mg/animal and 15–50 tumours/animal depending on the dose of Colo205 cells injected. The animals were sacrificed by cervical dislocation at the times indicated and the number of tumours and the tumour weight were determined. All tumours larger than 1 mg were counted. Each treatment cohort consisted of five or six animals to permit comparison to other treatment cohorts treated simultaneously with the same batch of effector cells.

Histochemistry of tumour

Tumour tissues were snap-frozen in isopentane, prechilled in liquid nitrogen. Cryosections (6 µm) were air-dried overnight and fixed in –20 °C acetone. The avidin-biotin-complex/horseradish-peroxidase technique was employed as the immunohistochemical method with diaminobenzidine as chromogenic substrate [6]. As primary antibodies C242 mAb (Pharmacia, Uppsala), CD3 mAb (Dakopatts, Glostrup, Denmark) and HLA-DR mAb (Beckton/Dickinson, Mountain View, Calif., USA) were used at a concentration of 5 µg/ml. Control stainings with an irrelevant mAb were negative (data not shown).

Results

Expression and binding of C242Fab-SEA

The plasmid pKP941, containing the gene encoding for the VH-CH1 domain of the C242 antibody heavy chain fused to

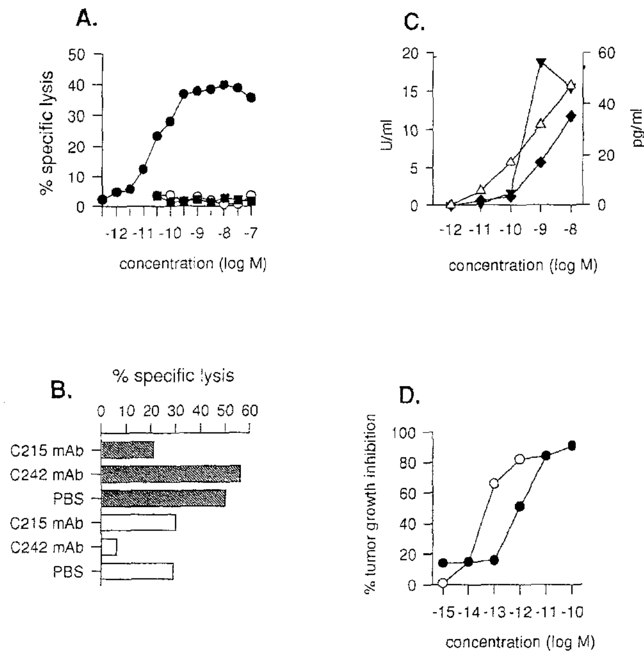

Fig. 2.A Dose response of C242Fab-SEA-dependent lymphocyte-mediated cytotoxicity of Colo205 tumour cells. Percentage lysis of ^{51}Cr-labelled Colo205 cells was determined after incubation for 6 h with preactivated T cells at an effector/target cell ratio of 50:1, with C242Fab-SEA (●), SEA (○) or C242Fab (■). **B** Specificity of the Fab-SEA-dependent cytotoxicity of Colo205 cells. Colo205 were incubated with 0.2 μM C242 or C215 mAb and with 10 nM C215Fab-SEA (*checked bars*) or 10 nM C242Fab-SEA (*open bars*) and with T cells at an effector/target cell ratio of 50:1 for 4 h. **C** Cytokine production in human peripheral blood mononuclear cells (PBM) induced by C242Fab-SEA fusion protein. Supernatants from day-3 cultures of PBM with C242Fab-SEA were analysed for interleukin-2 ($\times 10^3$ U/ml; ▼), interferon γ ($\times 10^2$ U/ml; ◆) and tumour necrosis factor α ($\times 10^2$ pg/ml; Δ). **D** Inhibition of tumour growth by C242Fab-SEA-induced cytokines. WiDr colon carcinoma cells were incubated with PBM and C242Fab-SEA (●) or SEA (○) at the concentrations indicated. After 6 days of culture the remaining viable tumour cells were recorded using a MTT assay. Data are given as percentage tumour growth inhibition

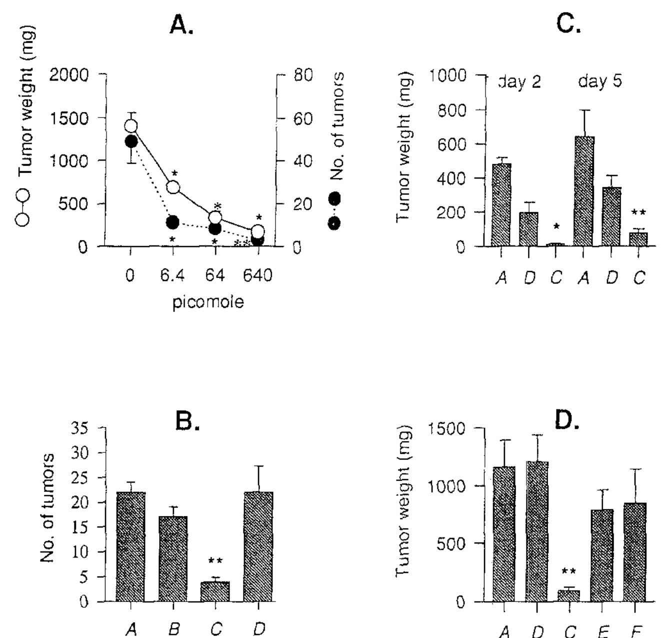

Fig. 3A–D Therapy of SCID mice xenografted i.p. with Colo205 colon carcinoma cells and human lymphocytes. **A** Dose dependence of therapy with i.v. injected C242Fab-SEA. SCID mice were injected i.p. with 7×10^6 Colo205 tumour cells (day 0), injected i.p. on day 1 with PBM and treated i.v. with varyious amounts of C242Fab-SEA on days 1, 3 and 6. The animals were sacrificed after 38 days, the tumours were counted and their weight determined. The effector/target cell ratio was 6:1. The values are the means ± SEM of five animals in each group. **B** Dependence of therapy on fusion protein and lymphocytes. SCID mice were injected i.p. with 10×10^6 Colo205 cells from tissue culture, injected i.p. on day 1 with PBM, treated i.v. with C242Fab-SEA (0.38 nmol/animal) on days 1, 3, 6 and 9 and sacrificed on day 37. The values are means ± SEM of five animals in each group. *A* Vehicle, *B* C242Fab-SEA, *C* PBM + C242Fab-SEA, *D* PBM. **C** Therapy of established disseminated tumours. SCID mice were injected i.p. with 5×10^6 Colo205 tumour cells 5 or 2 days before i.p. treatment with PBM and i.v. injections with C242Fab-SEA (0.38 nmol/animal). Two more injections of C242Fab-SEA were given at 3-day intervals. Examination on day 5 showed the macroscopic presence of multiple small tumours. The animals were sacrificed on day 27. The values are the means ± SEM of five animals in each group. *A* Vehicle, *C* PBM + C242Fab-SEA, *D* PBM. **D** Requirement of SEA and the C242Fab moieties for optimal therapy. SCID mice were injected i.p. with 3×10^6 Colo205 tumour cells and with PBM on day 1, and treated three times i.v. (every 3rd day) with vehicle or 0.38 nmol recombinant protein/animal. The effector/target cell ratio was 3:1. The animals were sacrificed after 34 days. The values are the means ± SEM of six animals in each group. *A* Vehicle, *C* PBM + C242Fab-SEA, *D* PBM, *E* PBM + C242Fab, *F* PBM + SEA. Statistical significance of treatment was determined using the Mann-Whitney *U*-test by comparing treated animals with animals receiving only PBM: * $P \leq 0.05$, ** $0.05 < P \leq 0.01$

the SEA gene, was expressed in a bicistronic transcription unit together with the VL-CL gene of the C242 antibody light chain (Fig. 1A, B). The Fab-SEA protein was produced by *E. coli* fermentation and purified to homogeneity as a 77-kDa fusion protein (Fig. 1B). The purified fusion protein demonstrated a preferential binding to the tumour antigen with a binding affinity (K_d) for the C242 tumour antigen of 28 nM (Fig. 1C) and for MHC class II molecules of about 151 nM (Fig. 1D).

Activation of cytotoxic and cytostatic T cell effector functions

To explore the antitumour effects of C242Fab-SEA, we studied antibody-targeted T-cell-dependent cytotoxicity [4] and secretion of tumour-suppressive cytokines in vitro [5]. C242Fab-SEA, but not native SEA or the C242Fab fragment, targeted T cells to lyse MHC class II$^-$ C242$^+$ human colon cancer cells (Fig. 2A). The cell killing was dependent on expression of the tumour antigen, since excess of C242 mAb but not the irrelevant C215 mAb blocked lysis (Fig. 2B).

SEA induces secretion of large amounts of cytokines, which suppress growth of colon cancer cells [5]. C242Fab-SEA retained the ability to induce cytokines in human PBM (Fig. 2C), which suppressed growth of colon cancer cells (Fig. 2D). The potency of the fusion protein to induce these

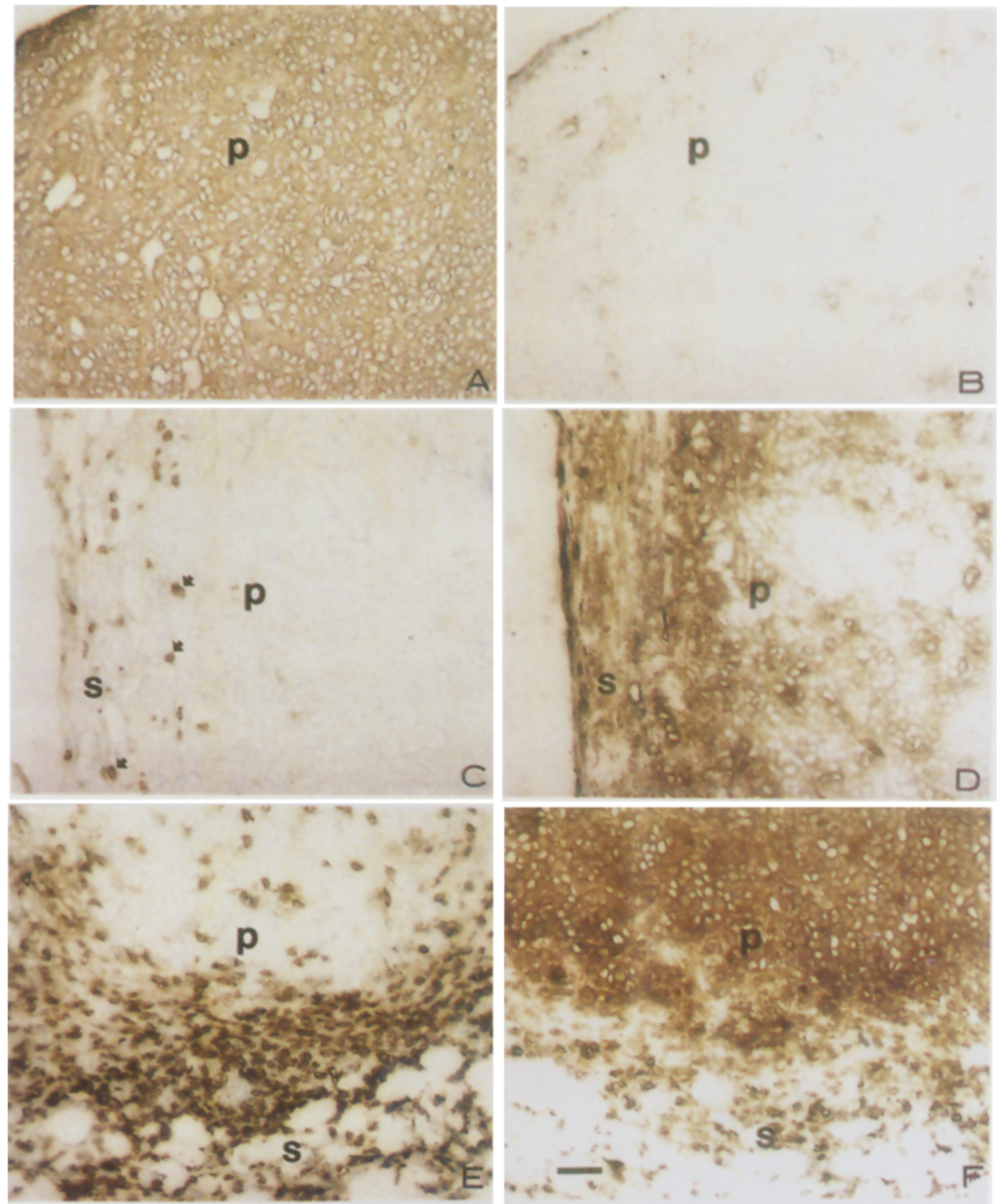

Fig. 4 A–F Immunohistochemical analysis of colon carcinoma from C242Fab-SEA-treated humanized SCID mice. **A, B** Tumour from an untreated animal stained with C242 mAb (**A**) and HLA-DR mAb (**B**). **C, D** Tumour from an animal grafted with human PBM and stained with CD3 mAb (**C**) and HLA-DR mAb (**D**). **E, F** Tumour from an animal grafted with human PBM, treated with C242Fab-SEA and stained with CD3 mAb (**E**) or HLA-DR mAb (**F**) *p, s* Tumour parenchyma and stroma respectively; *bar* denotes 50 μm and is valid for **A–F**

MHC-II-dependent activities was about ten times less effective than that of native SEA, which correlates with the lower MHC II binding affinity in the fusion protein compared to SEA (Fig. 1 C, D).

To study antitumour effects in vivo, we used a tumour model employing SCID mice xenografted i. p. with human colon cancer cells and human lymphocytes [15]. Repeated intravenous injections of C242Fab-SEA strongly reduced both tumour mass and number (Fig. 3 A). A substantial fraction of treated animals (15%–20%) were tumour-free 30–40 days after treatment. Effective therapy was seen only in animals receiving both C242Fab-SEA and human PBM (Fig. 3 B). A strong antitumour effect was also seen in animals carrying intraperitoneal disseminated, macroscopically detectable tumours (day-5 tumours, Fig. 3 C), indicating that the therapy may be effective in conditions with minimal residual tumour disease as well as in more advanced disease. Tumour targeting by the fusion protein was required for the antitumour effect since neither SEA nor C242Fab alone (Fig. 3 D) gave any significant tumour therapy.

Immunohistochemical analysis of the C242 and HLA-DR antigen expression in biopsies from untreated tumours revealed the $C242^+$ tumours to be HLA-DR-negative (Fig. 4 A, B). Injection of PBM showed an accumulation of $CD3^+$ cells in the stroma of the tumour and a moderate induction of HLA-DR on the outer margin of the tumour (Fig. 4 C, D). The observed reactivity with C242, CD3 and HLA-DR mAb was specific since no staining was seen with an irrelevant control mAb (data not shown). Therapy with PBM+C242Fab-SEA induced massive infiltration of $CD3^+$ cells and strong expression of HLA-DR in the entire tumour parenchyma (Fig. 4 E, F). Both $CD4^+$ and $CD8^+$ cells were seen (data not shown).

Discussion

In this paper we have shown that targeted superantigens are effective in immunotherapy of human colon cancer. Binding of C242Fab-SEA to tumour cells is likely to be effective in targeting cytotoxic T cells to antigen-positive tumour cells. However, in order to eliminate antigen-negative bystander tumour cells, release of cytokines in the tumour area may be important. In contrast to other antibody-based T cell therapies, such as bifunctional mAb reacting with the TCR-CD3 complex and a tumour-associated antigen [12], targeted superantigens possess several advantages. (a) Fab-SEA protein does not interact with T cells unless presented on a surface [6]. (b) Superantigen-induced activation of T cells leaves the majority of the T cell repertoire naive, preserving normal immune surveillance against infectious agents. (c) Fab-SEA fusion proteins are produced as recombinant single proteins and are easily purified to a high yield. (d) Fab-SEA is more potent than any other known T cell mitogen [14]. Indeed, a recent comparison of a mAb-SEB chemical conjugate and an anti-TCR-SEB bifunctional mAb in a mouse lymphoma model favoured the use of the mAb-superantigen in tumour therapy [11]. The ability of the colon-carcinoma-reactive superantigen to induce a strong T cell attack on established micrometastases implies that tumours considered beyond the reach of immunotherapy are treatable. C242Fab-SEA-induced T cell infiltration coincided with induction of HLA-DR and ICAM (data not shown), on the tumours, which possibly relates to local release of cytokines. Indeed, staining with IFNγ and TNFα mAb revealed infiltration of numerous cytokine-producing T cells after C242Fab-SEA therapy (M. Litton et al., manuscript in preparation). These findings are relevant for the therapy of human tumour since human colon cancers are commonly infiltrated by lymphocytes, may express HLA-DR and are sensitive to superantigen-induced cytotoxicity and cytokines in vitro [5, 8]. Induction of MHC class II and ICAM-1 in C242Fab-SEA-treated tumours further favours the subsequent response of cytotoxic and helper T cells [3]. Immunohistochemistry revealed that Fab-SEA therapy induced massive T cell infiltration in the periphery of the tumour and a significant but moderate infiltration in the centre of the tumour. In contrast, induction of HLA-DR and ICAM-1 on the tumour cells was prominent throughout the entire tumour parenchyma. This suggests that Fab-SEA-induced proinflammatory cytokines may diffuse in the tumour area and affect surrounding bystander tumour cells. It is tempting to suggest that such inflammatory effects may serve as an important therapeutic mechanism against tumours heterogeneous in antigen expression. The C242 antigen is well expressed in the majority of human colon cancers with limited expression in normal tissues, mainly in scattered cells in the colon [9]. The C242Fab-SEA fusion protein showed a similar immunohistochemical staining of human colon cancer tissues to the native C242 mAb (data not shown). This makes C242Fab-SEA a promising candidate for clinical immunotherapy in patients suffering from colon cancer micrometastases. In contrast, the C215Fab-SEA fusion protein [6], used as a control fusion protein in the present study, stains all human epithelial cells, which limits its use to experimental models.

Clinical experience with IL-2 immunotherapy has revealed that the balance between the effective biological dose and maximal tolerable dose may be narrow. C242Fab-SEA therapy is expected to improve this by favouring local versus systemic immune activation. C242Fab-SEA dose-escalation studies in primates have clearly shown that there is a distinct dose at which one can induce substantial immune activation, recorded as enhanced IL-2 serum levels, without toxic side-effects (unpublished data). Since primates express the C242 antigen in the gastrointestinal tract to a similar extent to humans, the results suggest that biologically active doses of C242Fab-SEA are well tolerable.

At present there is a continuous uncovering of the role of superantigens in various pathogenic processes including viral infectivity, bacterial immune escape, autoimmune diseases, septic shock and immunodeficiency. The present study encourages a genetic approach to modify superantigens for therapy of human malignant disease.

Acknowledgements The contribution of Drs. T. Moks and L. Strandberg and the assistance of C. Hofvander, M. Lassen, C. Modéus, C. Nordenberg, B.-M. Johansson and Dr. G. Andersson is greatly appreciated.

References

1. Björk P, Jönsson U, Svedberg H, Larsson K, Lind P, Dillner J, Hedlund G, Dohlsten M, Kalland T (1993) Isolation, partial characterization and molecular cloning of a human colon adenom carcinoma cell-surface glycoprotein recognized by the C215 mouse monoclonal antibody. J Biol Chem 268: 2432
2. Chen L, Ashe S, Brady WA, Hellstrom I, Hellström KE, Ledbetter JA, McGowan P, Linsley PS (1992) Costimulation of antitumor immunity by the B7 counterreceptor for the T lymphocyte moleuces CD28 and CTLA-4. Cell 71: 1093
3. Dohlsten M. Hedlund G, Lando PA, Trowsdale J, Altmann D, Patarroyo M, Fischer H, Kalland T (1991) Role of the adhesion molecule ICAM-1 (CD54) in staphylococcal enterotoxin mediated cytotoxicity. Eur J Immunol 21: 131
4. Dohlsten M, Hedlund G, Åkerblom E, Lando PA, Kalland T (1991) Monoclonal antibody-targeted superantigens: a different class of anti-tumour agents. Proc Natl Acad Sci USA 88: 9287
5. Dohlsten M, Sundstedt A, Björklund M, Hedlung G, Kalland T (1993) Superantigen-induced cytokines suppress growth of human colon-carcinoma cells. Int J Cancer 54: 482
6. Dohlsten M, Abrahmsén L, Björk P, Lando PA, Hedlund G, Forsberg G, Brodin T, Gascoigne NR, Förberg C, Lind P, Kalland T (1994) Monoclonal antibody-superantigen fusion proteins: Tumour-specific agents for T-cell-based tumor therapy. Proc Natl Acad Sci USA 91: 8945
7. Fischer H, Dohlsten M, Andersson U, Hedlund G, Ericsson P-O, Hansson J, Sjögren H-O (1990) Production of TNFα and TNFβ by staphylococcal enterotoxin A activated human T cells. J Immunol 144: 4663
8. Lando P, Dohlsten M, Hedlund G, Åkerblom E, Kalland T (1993) T cell killing of human colon carcinomas by monoclonal-antibody-targeted superantigens. Cancer Immunol Immunother 36: 223

9. Larson LN, Johansson C, Lindholm L, Holmgren H (1988) Mouse monoclonal antibodies for experimental immunotherapy promotes killing of tumour cells. Int J Cancer 42: 877
10. Momburg F, Ziegler A, Harpprecht J, Möller P, Moldenhauer G, Hammerling GJ (1989) Selective loss of HLA-A or HLA-B antigen expression in colon carcinoma. J Immunol 142: 352
11. Ochi A, Migita K, Xu J, Siminovitch K (1993) In vivo tumor immunotherapy by a bacterial superantigen. J Immunol 151: 3180
12. Renner C, Jung W, Sahin U, Denfeld R, Pohl C, Trumper L, Hartmann F, Diehl V, Lier R van, Pfreundschuh M (1994) Cure of xenografted human tumours by bispecific monoclonal antibodies and human T cells. Science 264: 833
13. Riethmüller G, Johnson JP (1992) Monoclonal antibodies in the detection and therapy of micrometastatic epithelial cancers. Curr Opin Immunol 4: 647
14. Scherer MT, Ignatowicz L, Winslow G, Kappler J, Marrack P (1993) Superantigens: bacterial and viral proteins that manipulate the immune system. Annu Rev Cell Biol 9: 101
15. Torbett BE, Picchio G, Mosier DE (1991) hu-PBL-SCID mice: a model for human immune function, AIDS, and lymphomagenesis. Immunol Rev 124: 139
16. Yoo Y-K, Heo DS, Van Thiel DH, Whiteside TL (1990) Tumor-infiltrating lymphocytes from human colon carcinomas. Functional and phenotypic characteristics after long-term culture in recombinant interleukin 2. Gastroenterology 98: 259