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Original Paper

Development of Immunogenic Colorectal Cancer Cell Lines for Vaccination: Expression of CD80 (B7.1) is not Sufficient to Restore Impaired Primary T Cell Activation *In Vitro*

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The capacity of colorectal carcinoma and melanoma cell lines to induce primary versus effector T lymphocyte activation *in vitro* was investigated. Established epithelial tumour cell lines derived from colorectal carcinoma and melanoma did not activate a primary proliferative response of resting T lymphocytes in allogeneic mixed lymphocyte tumour cell cultures (MLTCs). In contrast, the same tumour cells were effectively lysed by pre-activated cytolytic T cell clones. This demonstrates that tumour cells are impaired in inducing a primary immune response but are susceptible to effector immune responses. Attempts at improving primary T cell activation revealed that exogenous cytokines, including interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and interleukin-2 (IL-2), were not effective. Expression of CD80 (B7.1), by transfecting a CD80 cDNA into the melanoma cell line SkMel63, improved T cell proliferation considerably. In contrast, CD80 expression in two colorectal carcinoma cell lines (SW480, SW707) did not result in T cell activation. This was not due to lack of class II MHC expression on SW480 since coexpression of a HLA-DR3 alloantigen and CD80 had no effect. Our data suggest that *de novo* CD80 expression is not, in general, sufficient to improve primary T cell activation by human tumour cells.

Key words: adhesion molecules, colon carcinoma, melanoma, cytotoxic T cells, lymphocyte activation
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INTRODUCTION

THERE IS now increasing evidence that tumour cells express tumour-specific antigenic peptides which associate with MHC molecules and, therefore, can potentially stimulate a tumour-specific T cell response. These peptide antigens can be derived from tumour associated viruses, from products of mutated genes present only in tumour cells, or from unaltered proteins which are preferentially overexpressed [1–3]. Antigens derived from cellular proteins of human tumours have been characterised for melanomas [4–7].

According to a current model of T cell activation (two signal hypothesis), costimulatory signals (besides the T cell receptor-(TCR-) mediated signal following encounter of peptide/MHC-complex) are required for optimal T cell activation [8]. Using monoclonal antibodies (MAb) [9–11], soluble ligand variants [12, 13] and *de novo* expression of ligands [14, 15], it has been demonstrated that binding of T cell accessory receptors CD2, CD11/18 and CD28 to their respective cellular ligands/coreceptors CD58, CD54 and CD80 (B7.1) provide secondary signals required for T cell activation. Moreover, the presence of ligands for T cell accessory receptors (e.g. CD80) by antigen-presenting cells has been shown to be critical for the prevention of T cell anergy [16].

Tumour cells are not "professional" antigen-presenting cells. They generally do not express class II MHC molecules, and expression of costimulatory/accessory molecules is reduced or completely lost. For example, expression of CD80 is restricted to lymphoid tissue and, thus, is not found on epithelial cells [17]. In addition, active downregulation of class I MHC molecules and accessory molecules, such as CD58 and CD54, on tumour cells has been described and may contribute to immune escape [18, 19]. The fact that tumour cells do not deliver costimulatory signals would explain why, despite the presence of recognisable antigens, tumour cells do not stimulate an immune response but

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rather induce a state of specific tolerance. Therefore, expression of genes encoding cytokines or costimulatory molecules in tumour cells might improve their capacity to induce immune responses. In this regard, expression of interleukin-2 (IL-2), IL-4, interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor and CD80, respectively, have been shown to induce tumour rejection in murine tumour models [20–26], and represent promising approaches towards enhancing immune reactions against human tumours.

We have investigated the capacity of colorectal carcinoma and melanoma cell lines to induce primary versus effector T lymphocyte activation *in vitro*. Since primary activation of T cells is impaired and not augmented by exogenous cytokines such as IFN-γ, TNF-α and IL-2, respectively, we tested the effect of providing a costimulatory molecule by *de novo* expression of CD80. Our results demonstrate that CD80 expression restores impaired primary T cell activation of a human melanoma but not that of two colorectal carcinoma cell lines.

MATERIALS AND METHODS

Cell lines

The colorectal carcinoma cell lines SW480 and SW707 (culture medium: RPMI, Gibco, Eggenstein, Germany) were obtained from the European Collection of Animal Cell Cultures (Salisbury, U.K.) and the DKFZ Tumorbank (Heidelberg, Germany), respectively. The melanoma cell lines MzMe19 and SkMe163 (Dulbecco's Modified Eagles Medium [DMEM], Gibco) were kindly provided by Dr A. Knuth (Nordwestkrankenhaus, Frankfurt/Main, Germany). Media were supplemented with 10% fetal calf serum (FCS) (Serva, Heidelberg, Germany), 2 mM L-glutamine (Gibco) and 1% penicillin/streptomycin (Gibco). M7 and EB2 are Epstein–Barr virus-transformed B-lymphoblastoid cell lines (B-LCLs). T lymphocytes were purified from peripheral blood mononuclear cells (PBMC, prepared by density centrifugation) after adherence on plastic dishes and subsequent rosetting with sheep erythrocytes. T cells were > 90% $CD3^+$, > 95% $CD2^+$ and < 1% $CD14^+$. HLA-A2 restricted cytolytic T lymphocyte (CTL) clones, specific for an Influenza type A virus matrix protein-derived peptide (amino acids 57–68, MP57–68), were generated by stimulating PBMC from HLA-A2 positive donors with 5 μg/ml of the synthetic peptide KGILGFVFTLTV (single letter amino acid code) as previously described [27]. T cells were cloned by limiting dilution, and further expanded by restimulation in the presence of 20 U/ml IL-2 (Biotest, Dreieich, Germany) every 21 days with PBMC (6×10^4 cells/well, 96-well V-bottomed microtitre plate) and autologous B-LCLs (4×10^4 cells/well) previously pulsed with 10 μg/ml peptide. A HLA-DR3-reactive T cell line was generated following stimulation of PBMC of a HLA-$DR3^-$ donor with a HLA-$DR3^+$ B-LCL (kindly provided by Dr D. Kabelitz, Paul Ehrlich Institut, Langen, Germany). T cells were subsequently restimulated with two different HLA-$DR3^+$ B-LCLs and propagated by weekly stimulations with these B-LCLs in the presence of 20 U/ml IL-2.

Transfections

For B7.1-transfection, tumour cells were electroporated with 10 μg of the ScaI linearised plasmid pcEXV-B7 (kindly provided by Dr M. Jenkins, University of Minnesota, Minneapolis, Minnesota, U.S.A.) [28], containing a B7.1 cDNA and the neomycin resistance gene. Electroporation was performed at 100 Ω, 960 μF, 340 V (SW480, SW707) and 280 V (SkMel63), respectively, in HEPES balanced salt (HBS)-buffer (21 mM HEPES, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4, 6 mM glucose, pH 7.1, 4°C) using 4 mm cuvettes and a Gene Pulser (BioRad, Munich, Germany). Electroporated cells were plated at 10^5 cells/ml in 24-well plates. For HLA-DR3-transfection, 4×10^6 tumour cells/10 cm dish were cotransfected with 10 μg of the NdeI linearised plasmids pFM306 (DRα) and pFM307 (DRβ3) using 20 μl lipofectin (Gibco) under conditions provided by the manufacturer. pFM306 and pFM307 were constructed by Dr F. Momburg (DKFZ, Heidelberg, Germany) by inserting DRα and DRβ3 cDNA, respectively, obtained from pBGN-DRα and a pBGN-DRβ3 (kindly provided by Dr B. Mach, Geneva, Switzerland), together with a polylinker from pSP72 (Promega, Heidelberg, Germany) into pHβAP-1-neo [29]. SW480A6 cells were similarly cotransfected with pFM306, pFM307 and a plasmid containing the hygromycin resistance gene (pX343). Selection with G418 (Gibco, 1 mg/ml) and hygromycin (Boehringer Mannheim, Mannheim, Germany, 300 μg/ml) was started 48 h following transfection.

Immunofluorescence

Indirect immunofluorescence was performed as described [9]. MAbs were W6/32 (anti-class I MHC), BB7.2 (anti-HLA-A2), MEM112 (anti-CD54, Dr V. Horejsi, Prague, Czech Republic), AICD58.5 (anti-CD58, our laboratory), MAb104 (anti-CD80, Dianova, Hamburg, Germany), AIMHCII (anti-class MHC, our laboratory), and a fluoresceinated rabbit anti-mouse-Ig ($F(ab')_2$) (Dako, Hamburg, Germany) as secondary antibody. Negative control samples were incubated with the secondary antibody alone. Fluorescence was measured using a Coulter Profile flow cytometer (Coulter Electronics, Hialeah, Florida U.S.A.) with logarithmic amplification (3 log scale). Histograms were analysed using Flow-software by G. Futterman (DKFZ).

Cytotoxicity assay

Cell mediated cytotoxicity was performed in a standard chromium release assay. MP57–68-specific T cells were incubated for 3 h with labelled tumour cells in the presence of different concentrations of the synthetic peptide. Specific cytotoxicity was calculated according to:

$$\frac{[\text{experimental cpm} - \text{spontaneous cpm}]}{[\text{maximal cpm} - \text{spontaneous cpm}] \times 100}$$

Proliferation assay

T cells were cultured at 10^5 cells per well in 96-well flat bottomed microtitre plates (NUNC, Roskilde, Denmark) for 5 days, together with 10^4 stimulator cells, which were pretreated with 5 μg/ml mitomycin C (Sigma, Munich, Germany) for 2 h at 37°C and extensively washed. Cytokines were added at 500 U/ml (IFN-γ), 25 ng/ml (TNF-α) and 10 U/ml (IL-2), respectively. The CD28 MAb (Dr V. v. Fliedner, Ludwig Institute, Epalinges, Switzerland) was of IgM isotype and comitogenic in soluble form without crosslinking; it was used as culture supernatant at a final concentration of 25%. CD3 MAb (OKT3, 10 ng/ml) was immobilised on 96-well plates precoated with a rabbit anti-mouse antiserum (Dako, Hamburg, Germany, 30 μg/ml). Phorbol-12-myristate-13-acetate (PMA, Sigma) was used at 10^{-8} M, the CD80-specific MAb (MAb 104) at 2 μg/ml. Following addition of 37 kBq [^{3}H] thymidine (74.0 GBq/mmol, New England Nuclear, Boston, Massachusetts, U.S.A.) per well, cells were incubated for a further 18 h. Thymidine uptake was

Table 1. Analysis of T cell proliferation in allogeneic MLTC

Responder cells	Stimulator cells: None	SW480	SW707	MzMel9	SkMel63	M7
None	150*	319	420	2556	496	180
Donor 1: T cells	191	188	235	3052	1093	27725
Donor 2: T cells	366	258	213	4161	2212	27016
T cells + M7	27725	22746	21120	25433	25005	nd
T cells + IFN-γ	310	147	265	853	1242	nd
T cells + TNF-α	290	426	516	2374	2596	nd
T cells + IL-2	1817	3138	2542	4613	4866	nd

* [3H] thymidine incorporation (cpm). nd, not done.

determined by liquid scintillation counting and expressed as mean cpm of triplicate wells.

RESULTS

Epithelial tumour cell lines do not stimulate resting T lymphocytes in an allogeneic MLTC

Established epithelial tumour cell lines from colorectal carcinoma (SW480, SW707) and melanoma (MzMel9, SkMel63) were investigated for their capacity to activate T lymphocytes *in vitro* by incubating them with allogeneic peripheral blood T lymphocytes of healthy donors. In this situation, T lymphocytes should recognise class I MHC alloantigens and respond with proliferation. Table 1 shows the result of one representative experiment with T cells from two different donors, demonstrating that no substantial T lymphocyte proliferation was induced by tumour cells. In marked contrast, T cell proliferation was promoted by the allogeneic B-LCL M7 under the same conditions. The inability of tumour cells to stimulate T cell proliferation was independent of the T cell donor employed, and was observed with additional cell lines from melanoma and colorectal cancer as well as with cell lines from other tissues (data not shown). To eliminate the possibility that tumour cells actively inhibited T cell proliferation, we stimulated T cells in the presence of tumour cells with the B-LCL M7. As shown in Table 1, T lymphocyte proliferation was not blocked by any tumour cell line tested.

Phenotypic characterisation of tumour cell lines demonstrated that significant levels of class I MHC molecules were present on the cell surface, which potentially could serve as alloantigens (Table 2). Note that all cell lines expressed variable levels of CD58, both melanoma cell lines expressed CD54, and SkMel63 was also positive for class II MHC.

Treatment of tumour cells with IFN-γ and TNF-α, respectively, resulted in 2- to 3-fold increased levels of class I MHC on all four tumour cell lines as well as in *de novo* CD54 expression on SW480 and SW707 (data not shown). In order to test the effect of this increased class I MHC and CD54 expression, IFN-γ and TNF-α, respectively, were added to MLTC experiments. Table 1 shows that neither cytokines changed the poor capacity of any cell line to stimulate allogeneic T cell proliferation. Even IL-2 was not able to promote substantial tumour cell dependent T cell proliferation. Similar results were obtained when T lymphocytes from different donors were employed, and when tumour cells were pretreated for 24 h with IFN-γ or TNF-α prior to T cell stimulation.

Table 2. Immunofluorescence analysis of tumour cell lines

Cell line	Neg.	Class I MHC	Class II MHC	CD54	CD58
SW480	3.1*	74.3	3.0	3.2	19.8
SW707	2.8	98.3	3.2	3.3	14.8
MzMel9	2.8	27.2	2.9	8.0	5.6
SkMel63	2.0	33.6	18.8	12.8	4.2
M7	2.7	353.3	86.8	7.5	17.5

* Mean fluorescence intensity. Neg., negative control.

Tumour cell lines are susceptible to cytolytic T lymphocyte effector function

In order to test whether tumour cell lines induce cytolytic effector functions of activated T lymphocytes, we generated long-term human T cell clones specific for an antigenic epitope of the Influenza type A virus matrix protein. Such T cell clones are $CD8^+$, express α/β T cell receptors and specifically react with a synthetic peptide derived from the matrix protein (MP57–68) when presented in association with HLA-A2 molecules (data not shown). HLA-A2 positive epithelial tumour cells were then employed as target cells in cytotoxicity assays. As shown in Figure 1, the colorectal carcinoma cell lines SW480 and SW707 as well as the melanoma cell lines MzMel9 and SkME163 were

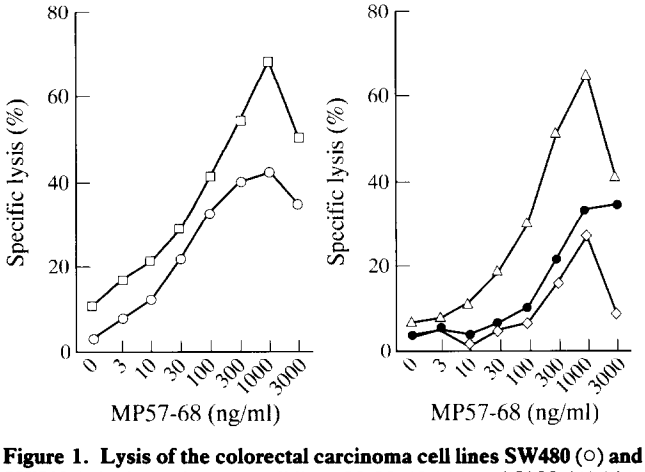

Figure 1. Lysis of the colorectal carcinoma cell lines SW480 (○) and SW707 (□), the melanoma cell lines MzMel9 (△) and SkMel63 (◇), and the B-LCL EB2 (●), respectively, by T cell clone U29.1, assessed at effector/target ratios of 10:1 and different concentrations of the peptide, MP57–68.

specifically and dose dependently lysed by the T cell clone U29.1, following addition of peptide MP57–68. Similar results were obtained employing a second MP57–68-specific T cell clone (not shown). Interestingly, cytolysis of tumour cells was similar if not higher when compared with the HLA-A2-positive B-LCL EB2 and occurred at comparable peptide concentrations, yielding half maximal lysis in the range of 0.1 to 1.0 μg/ml (Figure 1). Cytolysis was slightly reduced at peptide concentrations exceeding 1 μg/ml as reported before by others [27].

Effect of CD80 expression on tumour cell-induced primary T cell activation

To test the effect of CD80, known to provide strong costimulatory signals, a CD80 cDNA was transfected into two colorectal carcinoma cell lines (SW480, SW707) and one melanoma cell line (SkMel63). Figure 2 shows that CD80 cell surface levels of at least one clone derived from each of the tumour cell lines was comparable to that typically found on a constitutive CD80 positive B-LCL (M7). CD80 expression on transfected tumour cells was found to be stable for a period of at least 6 months and expression of MHC molecules, CD54 and CD58, was unchanged when compared with non-transfected cells (data not shown). CD80-transfected tumour cells were then tested for their ability to induce proliferation of resting allogeneic T lymphocytes. As shown in Figure 3(a), three different CD80-transfected SkMel63 clones promoted increased T cell proliferation (2.5- to 6-fold) in comparison with untransfected SkMel63. Although SkMel63A15 expressed low CD80 cell surface levels, this clone effectively stimulated T cell proliferation, indicating that a low threshold of CD80 expression was sufficient to exhibit functional effects. In contrast to SkMel63 transfectants, CD80 expression on SW480 and SW707 had no or very little effect on T cell mitogenesis. The weak stimulation by SW707A33 shown in Figure 3(a) was the strongest response observed. In the majority of experiments, however, colorectal carcinoma cell lines were not found to initiate T cell mitogenesis following CD80 transfection. Figure 3(b) shows that all CD80-transfectants of SW480 and SW707 promoted T cell proliferation in conjunction with submitogenic concentrations of CD3 MAb (OKT3) or PMA, indicating that CD80 molecules expressed on these cells were functionally active. Note that in addition to the experiment described here, clones SW480A7 and SW480A8 showed significant costimulation in combination with OKT3 or PMA in other experiments.

T cell proliferation induced by CD80-transfected SkMel63 was specifically inhibited by a CD80 MAb as shown in Figure 3(c). This verifies that growth promotion observed with these clones was a result of their CD80 expression. Similar to the *de novo* expression of CD80 on SkMel63 cells, the addition of a comitogenic IgM CD28 MAb, which did not require crosslinking, increased the proliferative response of T lymphocytes towards SkMel63 (Figure 3d). In contrast, stimulation of T cells with SW480 or SW707 in conjunction with CD28 MAb did not lead to increased proliferation, which is in line with the negative results obtained with CD80 transfectants of these two colorectal carcinoma cell lines.

Expression of HLA-DR3 in combination with CD80 is not sufficient to modify the immunogenicity of SW480 cells

One phenotypic difference between SkMel63 and the two colorectal carcinoma cell lines was that SkMel63 cells expressed class II MHC molecules whereas SW480 and SW707 did not (Table 2). To eliminate the possibility that lack of class II MHC expression was responsible for the failure of these cells to stimulate T cells *in vitro*, even following CD80 expression, we transfected cDNAs encoding DRα and DRβ3 into SW480 and SW480A6 cells. Immunofluorescence analysis employing a MAb directed at monomorphic class II MHC epitopes (Figure 4) shows two clones expressing HLA-DR3 (SW480D13, SW480D19) and two clones coexpressing CD80 and HLA-DR3 (SW480A6D1, SW480A6D5). The level of HLA-DR3 expression on transfected cells was approximately three to four

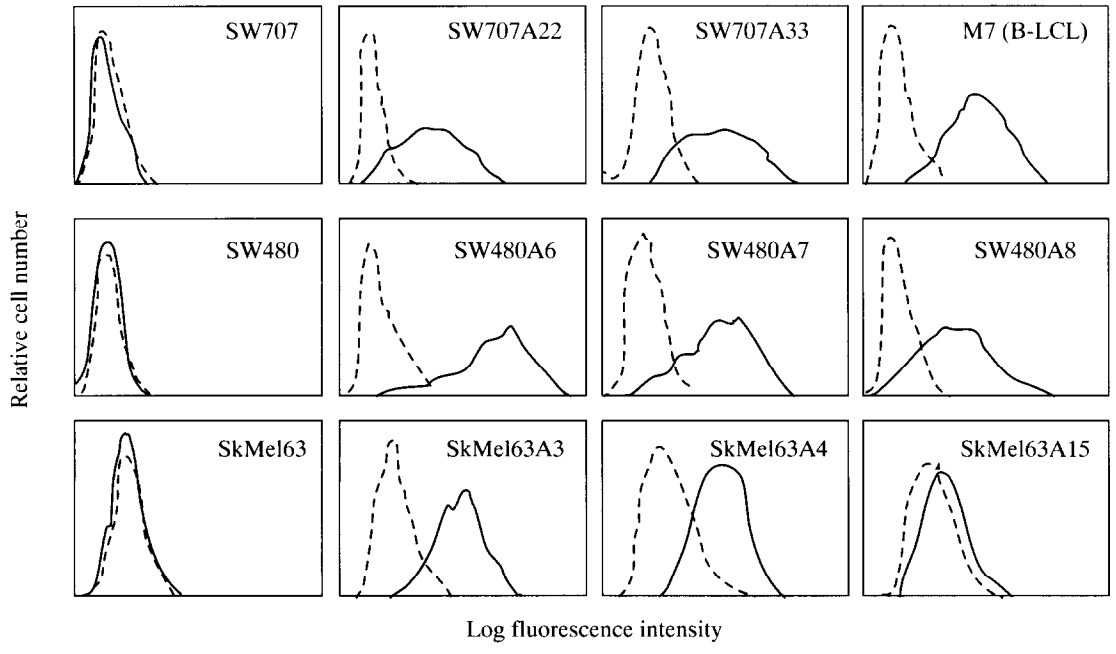

Figure 2. Cell surface expression of CD80 on individual clones of transfected tumour cell lines in comparison to the B-LCL M7. Determined by immunofluorescence analysis using labelled secondary MAb alone as negative control (broken lines) and CD80-specific (solid lines) MAb.

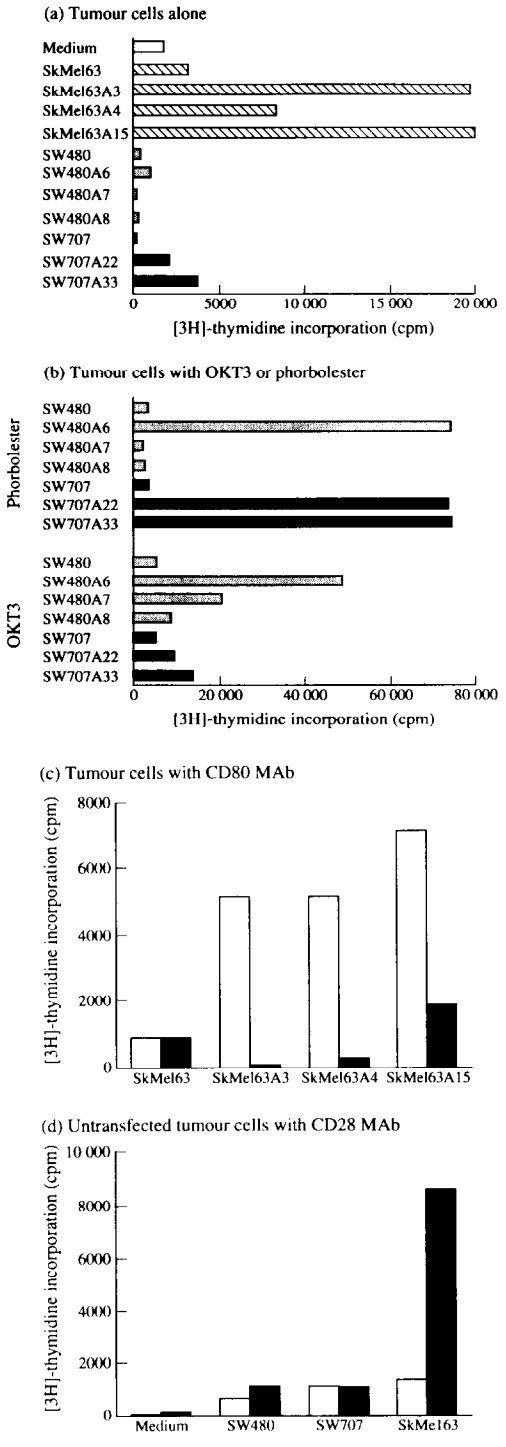

Figure 3. T cell proliferation in response to CD80-transfected tumour cells, determined in allogeneic MLTC experiments. (a) Proliferation of T cells in response to tumour cells alone. (b) Proliferation in response to tumour cells in combination with OKT3 and phorbolester, respectively. (c) Proliferation in response to tumour cells in the absence (open bars) or presence (solid bars) of a CD80-specific MAb. (d) Proliferation in response to untransfected tumour cells in the absence (open bars) or presence (solid bars) of a costimulatory CD28 MAb.

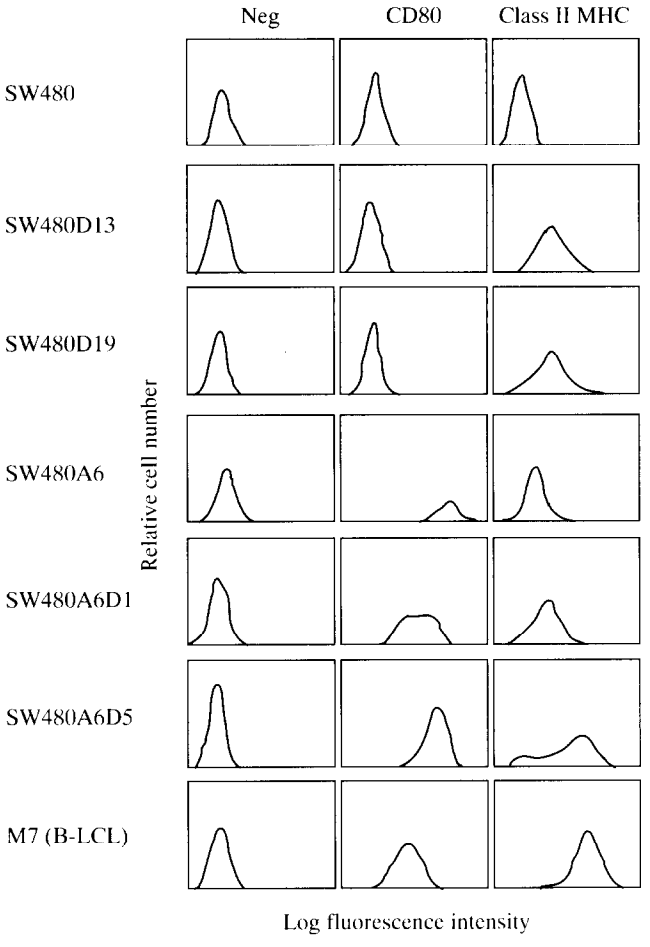

Figure 4. Cell surface expression of CD80 and the class II MHC allele HLA-DR3 on transfected SW480 cells and the B-LCL M7. Determined by immunofluorescence analysis using labelled secondary MAb alone as a negative control (Neg), CD80-specific, and class II MHC-specific MAb, respectively.

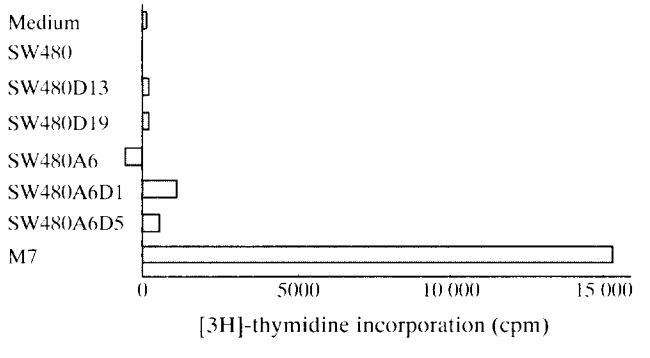

Figure 5. Effect of HLA-DR3 expression and CD80/HLA-DR3 coexpression, respectively, on T cell proliferation towards transfected SW480 cells determined in allogeneic MLTC.

times lower than the level of class II MHC molecules found on the B-LCL M7 (Figure 4), but comparable to class II MHC expression on SkMel63 (not shown).

We tested HLA-DR3 transfected SW480 cells in MLTC experiments. As shown in Figure 5, expression of HLA-DR3 molecules and, most importantly, coexpression of HLA-DR3 and CD80 molecules had no effect on primary T cell activation by SW480. To test whether HLA-DR3 molecules expressed by SW480 variants were principally functional, we generated a HLA-DR3-reactive T cell line and employed HLA-DR3 transfectants in cytotoxicity assays. In contrast to untransfected

SW480 cells, SW480D15 and SW480D19 cells were specifically lysed (data not shown).

DISCUSSION

Vaccination of tumour patients with tumour cells may represent one approach to induce a specific anti-tumour immune response in an adjuvant setting. The use of tumour cells as vaccines has the advantage that several peptides derived from multiple altered genes, displayed at the cell surface in association with MHC molecules, can be utilised as antigens even when their precise nature is unknown. However, tumour cells per se are only weakly immunogenic and, therefore, need to be modified to support the induction of immune responses. One approach aimed at improving the immunogenicity is to provide tumour cells with costimulatory molecules necessary for T lymphocyte activation, which are usually not expressed on epithelial tumours. More recently, the application of genetically modified tumour cells, either autologous or allogeneic with shared HLA-alleles, has been initiated in clinical trials in patients with malignant melanoma and renal cell cancer [30]. In order to generate immunogenic variants of human colorectal cancer cells, we investigated the effect of expressing the costimulatory molecule CD80 in such cells.

Unmodified tumour cell lines from colorectal cancer and melanoma did not stimulate proliferation of resting T lymphocytes in MLTCs using allogeneic HLA molecules as antigens. The fact that T cells from different donors were employed together with tumour cell lines of different HLA background makes it unlikely that insufficient HLA mismatches existed to activate alloreactive T cells. Increasing class I MHC molecule expression by IFN-γ and TNF-α, respectively, was not sufficient to restore T cell proliferation, suggesting that alloantigen levels were not a critical parameter for activation. In addition, we provided evidence that active inhibition of T cell proliferation by tumour cells is highly unlikely. In contrast to primary T cell activation, the same tumour cells were effectively lysed by activated cytolytic T cell clones specific for an epitope of the Influenza A virus matrix protein (MP57–68), following addition of the synthetic peptide antigen. This demonstrates that tumour cells are impaired in inducing a primary T cell response, and indicates that modifications aimed at improving the immunogenicity of tumour cells should focus on the induction phase of an immune response. Furthermore, our findings suggest that once cytolytic tumour specific T cell precursors are activated, i.e. by appropriate vaccination, such cells are able to kill tumour cells. This view is supported by an independent study employing mouse tumour cells in an *in vivo* situation [31].

We employed allogeneic MLTC experiments to study the effect of CD80 expression on T cell activation, since this facilitates the investigation of the initiation phase of an immune reaction. The high frequency of alloreactive precursors allows a direct measurement of T cell activation without the elaborative determination of tumour antigen specific T lymphocyte frequencies. Furthermore, cell lines with unknown tumour antigens, such as colorectal cancer cell lines, can be studied. Tumour cell modifications with positive effects in MLTC experiments are predicted to be effective in inducing tumour specific T cell responses. This can be further established in autologous systems or, alternatively, in situations where certain HLA-alleles are shared between tumour cells and T cells.

As shown, transfection of CD80 into tumour cells enhanced the capacity of the melanoma cell line SkMel63 to stimulate proliferation of resting T lymphocytes directly. The difference in inducing primary versus effector T cell responses by SkMel63 cells might, therefore, be based on a different requirement for costimulatory signals between resting and activated T cells. It has been previously reported that CD80 transfection into haematopoietic cell lines rendered them capable of stimulating alloreactive $CD4^+$ T lymphocytes [28, 32]. Similarly, expression of CD80 on a monocytic leukaemia, a melanoma and an ovarian carcinoma cell line was reported to induce T cell activation in allogeneic situations [33]. In our experiments, CD80 expression on the two colorectal carcinoma cell lines, SW480 and SW707, was ineffective. This indicates that delivery of a CD80-mediated costimulus by human tumour cells is not sufficient to restore primary T cell activation. A similar notion was suggested following the analysis of CD80 expressing mouse tumour cell lines *in vivo* [34, 35]. The lack of costimulatory molecules besides CD80 might account for the observation that CD80-transfection was ineffective in some tumour cell lines. Preliminary results obtained with SW480 cells indicate that the expression of CD54 seems to be critical for the function of CD80 (M. Lindauer and associates, manuscript in preparation). Here, we investigated whether the expression of a class II MHC allele had an effect on the immunogenicity of the colorectal carcinoma cell line SW480. However, $CD80^+/DR3^+$ SW480 did not promote T lymphocyte activation. Assuming that expression of one allogeneic class II MHC allele is sufficient to stimulate T cells in MLTCs makes the requirement for class II MHC expression likely. In this regard, it has been shown that class II MHC expression on murine CD80-transfected tumour cell lines did not correlate with their rejection [35]. Furthermore, CD80 expression on class II MHC negative tumour cells is expected to provide a costimulatory signal for class I restricted $CD8^+$ T cells because CD28 is expressed on approximately 50% of resting peripheral blood $CD8^+$ T lymphocytes [36], and CTLA-4, the second receptor for CD80, is expressed on all T lymphocytes following activation [37]. This is supported by the previous finding that CD80 expressing melanoma cells induced proliferation of purified $CD8^+$ T lymphocytes [33].

Tumour cell lines with defects in class I MHC-associated antigen presentation and, therefore, impaired capacity to deliver TCR-mediated signals have been reported [38]. These cell lines were characterised by absent or reduced expression of class I MHC molecules. In contrast, the colorectal carcinoma cell lines employed in our studies showed significant class I MHC surface expression at comparable if not higher levels than SkMel63 cells. Moreover, the surface class I molecules seem to be native because they interacted with MAb W6/32, which recognises only complexes of class I heavy chain, β_2-microglobulin and peptide. In addition, experiments by others have demonstrated that SW480 cells are able to present intracellular antigens [38]. The lack of effect following expression of a class II MHC alloantigen (HLA-DR3) on SW480 cells also points towards a deficiency at the level of costimulation. However, augmentation of OKT 3 induced proliferation by CD80-transfected SW480 and SW707 cells may indicate that insufficient stimulation occurred by class I MHC alloantigens in the absence of OKT3. Whether class I MHC molecules expressed on the surface of the two colorectal carcinoma cell lines are indeed functionally impaired and whether this can be overcome, i.e. by providing additional costimulatory signals, needs to be investigated.

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