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Induction of Impaired Antitumor Immunity by Fusion of MHC Class II-Deficient Dendritic Cells with Tumor Cells

Yasuhiro Tanaka,2* Shigeo Koido,2* Masaya Ohana,‡ Chunlei Liu,‡ and Jianlin Gong3*‡

To dissect the role of Ag presentation through MHC class I and/or II pathways by dendritic cell (DC)-tumor fusion cells, we have created various types of DC-tumor fusion cells by alternating fusion cell partners. Fusions of MC38/MUC1 carcinoma cells with DC from wild-type (WT-DC), MHC class I knockout (IKO-DC), class II knockout (IIKO-DC), or class I and II knockout (I/IIKO-DC) mice created WTDC-fusion cells (FC), IKO-FC, IIKO-FC, and I/IIKO-FC, respectively. MHC class II- and MUC1-positive fusion cells were constructed by fusion of B16/MUC1 melanoma cells with IKO-DC (IKO/B16-FC). Immunization of MUC1 transgenic mice with $5 \times 10^5$ WTDC-FC, IKO-FC, IIKO-FC, or I/IIKO-FC provided 100, 91.7, 61.5, and 15.4% protection, respectively, against tumor challenge with MC38/MUC1 cells. In contrast, all mice immunized with irradiated MC38/MUC1 tumor cells or WT-DC developed tumors. One group of mice was immunized with $5 \times 10^5$ IKO/B16-FC and then challenged with B16/Ia+/MUC1 on one flank and MC38/MUC1 on the other flank. Immunization of these mice with IKO/B16-FC resulted in 100 and 78.6% protection against B16/Ia+/MUC1 and MC38/MUC1 tumor challenge, respectively. The antitumor immunity induced by immunization with IKO/B16-FC was able to inhibit the growth of MHC class II-negative tumor. In addition, in vivo results correlated with the induction of Ag-specific CTL. Collectively, the data indicate that MHC class II Ag presentation targeting activation of CD4 T cells is indispensable for antitumor immunity. The Journal of Immunology, 2005, 174: 1274–1280.

Effective Ag processing and presentation are crucial to antitumor immunity. Tumor cells express tumor Ags, but seldom induce effective antitumor immune response. This observation is probably related to the fact that tumor cells do not express essential costimulatory molecules and seldom express MHC class II molecules. Therefore, effort has been made to enhance tumor cell-based vaccine using genetically modified tumor cells. Immunization of mice with autologous class II- and/or B7-transfected tumor cells provides protection against challenge with wild-type tumor (1, 2) and mediates regression of established tumor (3). Alternatively, a strategy has been developed based on dendritic cells (DC), which are bone marrow-derived leukocytes bearing a characteristic veiled morphology that excel in Ag presentation and the initiation of primary immune responses (4–6). DC reside at the port of entry, take up exogenous Ags, and migrate to draining lymph nodes, where the Ags are presented to CD4 T cells through MHC class II pathways. In addition, DC are capable of initiating CD8 T cell response through a cross-presentation pathway. Exogenous Ags from tumor cells can be delivered to the cytosol, processed, and presented through an endogenous pathway (7–10).

Another evolving strategy is the use of DC fused to tumor cells (11). In this approach, tumor Ags are delivered to DC, processed, and presented through both MHC class I and II pathways in the context of costimulatory molecules. The fusion cells function like APCs with the ability to migrate to draining lymph nodes, where they interact with CD4 and CD8 T cells and induce potent antitumor immunity (12, 13). Coculture of human peripheral blood monocytes with DC-tumor fusion cells induces both CD4 and CD8 T cells (14, 15). However, the role of MHC class I-restricted or class II-restricted Ag presentation and the activation of CD4 and CD8 T cells in the antitumor immune responses are not well defined. In the present study, we created various types of DC-tumor fusion cells with intact or deficient expression of MHC class I or II molecules by using several kinds of DC and tumor fusion partners. The fusion cells were used in the prevention and treatment of tumors in MUC1 transgenic mice (MUC1.Tg). We observed differential impairment of antitumor immunity induced by fusions of DC from MHC class I and/or II knockout mice. Immunization with MHC class II-deficient DC-tumor fusion cells abolished the IFN-γ production of CD4 and CD8 T cells and the induction of CTL, and severely impaired antitumor immunity. These results indicate that MHC class II Ag presentation targeting activation of CD4 T cells is indispensable in antitumor immunity.

Materials and Methods

DC generation

DC were obtained from bone marrow cultures of C57BL/6 wild-type (WT), MHC class I knockout (β2m−/−) (16), MHC class II knockout (Abb−/−) (17), and MHC class I and II double-knockout (β2m−/−Abb−/−) (18) mice from Taconic Farms, using the method previously described (19). Briefly, bone marrow cells were flushed from long bones and further depleted of lymphocytes, granulocytes, B cells, and APCs by incubation with anti-CD8 (2.43), anti-CD4 (GK1.5), anti-B220 (RA3-3A1/6.1), anti-Ia (B21-2), and anti-Gr-1 (RB6-8C5) (American Type Culture Collection (ATCC)) Abs, followed by rabbit complement. The cells were cultured in RPMI 1640 medium supplemented with...
5% heat-inactivated FCS, 50 μM 2-ME, 1 mM HEPES (pH 7.4), 2 mM glutamine, 10 U/ml penicillin, 100 μg/ml streptomycin, and 20 ng/ml murine rGM-CSF (Sigma-Aldrich). On day 5 of culture, DC were purified by multiple steps of plating and collection of the loosely adherent population. After overnight culture, DC were harvested for phenotype analysis and fusion to MC38/MUC1 cells.

**Tumor cells**

Murine MC38 colon adenocarcinoma and B16 melanoma cells were stably transfected with a MUC1 cDNA, resulting in MC38/MUC1 (20, 21) and B16/MUC1 (22). The B16/MUC1 cells positive for MUC1, but negative for both MHC class I and II were selected and fused to B16 positive for MHC class II (B16/Ia+)

### RT-PCR

Lymph node cells (LNC) were isolated from mice immunized twice with WTDC-FC, IFO-FC, IFO-FC, and I/IIKO-FC or treated with PBS, and 106 sorted CD4 or CD8 T cells was extracted by TRIzol reagent (Invitrogen Life Technologies). Total RNA to cDNA was reverse transcribed using a poly(dT) oligonucleotide and SuperScript (Invitrogen Life Technologies). PCR was performed by amplifying cDNA with the following oligonucleotide primer (23): murine IL-2 (5'-GAGTCAAATCCAGAACATGCC-3') and 5'-GATTGAACTCAGGAGACATGGC-3'); IFN-γ (5'-CATTGGAAGCTTAAAGGTCTGCT-3' and 5'-CTCATGGGAAATGCCTTCTTGGC-3'); β-actin (5'-TGTAGTTGTTGGAAATGTCGCTGAG-3' and 5'-TTTGTAGTGCAAGACATTGTTAC-3') (Stratagene). PCR-amplified products were analyzed on a 2% agarose gel.

### 51Cr cytotoxicity assay

Spleens from MUC1.Tg mice immunized twice with WTDC-FC, IFO-FC, IFO-FC, and I/IIKO-FC were removed, and T cells were isolated into single cell suspensions for use as effector cells. The targets included MC38, MC38/MUC1, B16, B16/MUC1, and MCF-7 tumor cells. Briefly, tumor cells (1–2 × 106 cells) were labeled with 100–200 μCi of Na251CrO4 for 60 min at 37°C, followed by thorough washing to remove unincorporated isotope. T cells and tumor targets were resuspended in culture medium and then combined at various E:T ratios in 96-well V-bottom plates. The plates were centrifuged at 100 × g for 5 min to initiate cell contact and incubated for 5 h at 37°C with 5% CO2. After incubation, supernatants were collected and radioactivity was quantified in a gamma counter. Spontaneous release of 51Cr was determined by incubation of targets in the absence of effectors, and maximum or total release of 51Cr by incubation of targets in 0.1% Triton X-100. The percentage of specific release of 51Cr was calculated using the following: percentage of specific release = (experimental – spontaneous)/(maximum – spontaneous) × 100.

### In vivo tumor prevention

C57BL/6 MUC1.Tg mice (a kind gift from Dr. S. J. Gendler, Mayo Clinic, Scottsdale, AZ) that express MUC1 at a level similar to that found in humans (24) were used. Seven-week-old MUC1.Tg mice were immunized s.c. on days 0 and 7 with 5 × 103 WTDC-FC, IFO-FC, IFO-FC, and I/IIKO-FC calculated on the basis of fusion efficiency. WT-DC were used as a control. On day 14, the mice were challenged by s.c. injection in the flank with 5 × 105 syngeneic MC38/MUC1 (left side) and MC38 (right side) tumor cells. To determine the antitumor immunity induced by MHC class II-expressing, but class I-deficient fusion cells, groups of MUC1.Tg mice were immunized twice s.c. with 5 × 105 I/IIKO-FC. Control mice were immunized with IFO-DC or irradiated tumor cells. Seven days after the second vaccination, the mice were challenged with 5 × 103 MC38/MUC1 tumor cells (MHC class I and MUC1-positive) on the left flank and B16/Ia+/MUC1 tumor cells (MUC1 and MHC class II positive, but class I negative) on the right flank.

All of the mice were followed for 30 days. The size of tumor was determined by measuring perpendicular dimensions with a vernier caliper every 2–3 days. Tumors with a diameter of ≥3 mm were designated as positive. The mice were maintained in microisolator cages under specific pathogen-free conditions.

### Treatment of established pulmonary metastases

Pulmonary metastases were established by i.v. injection of 1 × 106 MC38/MUC1 tumor cells through the tail vein in 7-wk-old MUC1.Tg mice. Two days after the tumor inoculation, mice were immunized with 1 × 105 WTDC-FC, IFO-FC, IFO-FC, or WT-DC. The immunization was repeated on day 8. The mice were sacrificed 20 days after the last immunization. Pulmonary metastases were enumerated by counting after stains using the lungs with India ink (25).

### Statistical analysis

Statistical significance was analyzed using χ2 and Student’s t tests.

### Results

Characterization of various types of DC-tumor fusion cells

Constitutive deletion of the gene for β2m results in loss of expression of MHC class I in all nucleated cells (16), whereas mutation in the Aβ gene abrogates MHC class II expression in all APCs (17). Therefore, DC from the β2m−/−/Ab−/− double-knockout mice are devoid of expression of MHC class I and II molecules (18). To confirm these findings, the phenotype of various types of DC, DC-tumor fusion cells, and tumor cells was assessed by FACS analysis. MC38/MUC1 tumor cells expressed MUC1 and MHC class I molecules (Fig. 1A). DC isolated from WT, β2m−/−, and Ab−/− mice expressed MHC class I and II, class II, and class I
molecules (Fig. 1B), respectively. $\beta_m^{-/-}/\text{Abb}^{-/-}$ mice expressed no MHC molecules. However, MHC class I and/or II deficiency did not affect the expression of B7-2 on DC. Fusion of MC38/MUC1 with WT-DC resulted in coexpression of MUC1 with MHC class I and II molecules or B7-2 (Fig. 1C). Similar results were obtained with IKO-DC fused to MC38/MUC1, indicating that tumor-derived MHC class I molecules were expressed. In contrast, IIKO-DC or I/IIKO-DC fused with MC38/MUC1 led to the expression of MUC1 and MHC class I or B7-2 molecules, but not MHC class II molecules (Fig. 1C). These data indicate that the properties of fusion cells are dictated by their parent cells and that DC-tumor fusion cells deficient in MHC class II expression can be created by using DC from $\text{Abb}^{-/-}$ or $\beta_m^{-/-}/\text{Abb}^{-/-}$ mice as fusion cell partners.

**Stimulation of T cell proliferation compromised in IIKO-FC**

To determine the ability of fusion cells to stimulate T cell proliferation, the MLR assay was used. T cells from splenocytes of BALB/c mice cocultured with WT-DC or WTDC-FC proliferated vigorously (Fig. 2A, left upper panel). Coculture of these T cells with IKO-DC or IKO-FC also resulted in proliferation of T cells, albeit at a lower level (Fig. 2A, left lower panel). In contrast, impaired T cell proliferation was observed when T cells were cocultured with IIKO-DC or I/IIKO-DC or their fusion counterparts.

**FIGURE 1.** Characterization of DC and DC-tumor fusion cells (FC). Cells were double stained with FITC-conjugated anti-MUC1 mAb and PE-conjugated anti-MHC class I, anti-MHC class II, or anti-B7-2 mAb and analyzed by flow cytometry. A, MC38/MUC1 carcinoma cells. B, DC isolated from WT mice (WT-DC), $\beta_m^{-/-}$ mice (IKO-DC) Abb$^{-/-}$ mice (IIKO-DC), and $\beta_m^{-/-}/\text{Abb}^{-/-}$ mice (I/IIKO-DC). C, WTDC, IKO-DC, IIKO-DC, or I/IIKO-DC were fused to MC38/MUC1 cells to create WTDC-FC, IKO-FC, IIKO-FC, and I/IIKO-FC, respectively.

**FIGURE 2.** Proliferation and activation of T cells by DC-tumor fusion cells. A, Splenocytes were isolated from naive BALB/c mice and purified through nylon wool. T cells were cocultured with WT-DC (□) and WTDC-FC (○) (upper left panel); IKO-DC (□) and IKO-FC (○) (lower left panel); IIKO-DC (□) and IIKO-FC (○) (upper right panel); or II/IIKO-DC (□) and I/IIKO-FC (○) (lower right panel) at various fusion cell-T cell ratios. Irradiated MC38/MUC1 cells (E) and T cells (') were used as controls. After 5 days, cells were pulsed with 1 μCi/well [3H]thymidine and harvested on filters. Radioactivity was measured by liquid scintillation counting. Results were repeated in three separate experiments. B, LNC were isolated from MUC1.Tg mice immunized twice with 5 × 10$^5$ WTDC-FC, IKO-FC, IIKO/FC, I/IIKO-FC, or PBS injection. T-LNC were obtained through nylon wool purification, stained with FITC anti-CD4 mAb and PE anti-CD8 mAb, and sorted into CD4$^+$ and CD8$^+$ T cells by immunofluorescence cell sorting. Total mRNA was extracted from sorted CD4$^+$ and CD8$^+$ T cells and assessed for TCR-$\beta$, IL-2, IFN-$\gamma$, and $\beta$-actin mRNA synthesis by RT-PCR. Similar results were obtained in two separate experiments.
IFN-$\gamma$ abolished in CD4 and CD8 T cells primed by IIKO-FC

Cytokine production is the hallmark of T cell activation. To determine whether cytokine expression was affected in T cells primed by various types of DC-tumor fusion cells, we used RT-PCR to assess the cytokine mRNA levels of LNC isolated 7 days after the second immunization. Whereas sorted CD4 T cells from mice immunized with WTDC-FC or IKO-FC expressed IL-2 and IFN-$\gamma$ (Fig. 2B), the expression of IFN-$\gamma$ was abolished in CD4 T cells primed by IIKO-FC or I/IIKO-FC. The expression of IL-2 was also abolished in CD4 T cells primed by I/IIKO-FC. Similarly, IFN-$\gamma$ was detected in sorted CD8 T cells from mice immunized with WTDC-FC or IKO-FC. However, IFN-$\gamma$ was not detected in CD8 T cells primed by IIKO-FC or I/IIKO-FC. These results indicate that MHC class II-restricted Ag presentation affects downstream cytokine production of both CD4 and CD8 T cells. The secretion of IFN-$\gamma$ was abolished in T cells primed by MHC class II-deficient fusion cells, indicating the impairment of T cell activation.

Presentation of MHC class II Ag required for effective induction of CTL

CTL response against tumor cells was evaluated by standard $^{51}$Cr release assays to assess the effectiveness of immunization with various types of DC-tumor fusion cells. Immunization with WTDC-FC, IKO-FC, IIKO-FC, and I/IIKO-FC resulted in 52, 37, 26, and 16% CTL activity, respectively, against MC38/MUC1 tumor cells (Fig. 3A). CTL elicited by WTDC-FC or IKO-FC showed moderate killing against MUC1-positive B16/MUC1 melanoma cells (Fig. 3A). Interestingly, immunization with WTDC-FC or IKO-FC induced 40 and 27% CTL activity, respectively, against MC38 (Fig. 3A), the parent tumor cell of MC38/MUC1, indicating that fusion cells elicited CTL not only against MUC1, but also against unidentified tumor Ag in MC38. In contrast, there were no CTL induced by WTDC-FC against unrelated B16 melanoma cells or MUC1-positive human breast carcinoma cells (Fig. 3A). Similar results were obtained in a separate experiment (Fig. 3B). These results indicate that immunization with DC-tumor fusion cells induces Ag-specific polyclonal CTL. In addition, CTL activity was almost abolished when mice were immunized with DC-tumor fusion cells deficient in MHC class II molecules.

Suboptimal protection from tumor challenge resulted from lack of MHC class II Ag presentation

To determine the requirement for MHC class I and/or II Ag presentation in antitumor immunity induced by the fusion cells, MUC1.Tg mice were immunized twice with WTDC-FC, IKO-FC, IIKO-FC, and I/IIKO-FC or DC, and then challenged with MC38/MUC1 tumor cells on one flank and MC38 tumor cells on the other flank. Immunization with WTDC-FC, IKO-FC, IIKO-FC, and I/IIKO-FC or WT-DC resulted in 100, 91.7, 61.5, 15.4, and 0% protection, respectively, against MC38/MUC1 tumor challenge (Fig. 4A). The protection against MC38 was slightly lower. Immunization with WTDC-FC, IKO-FC, IIKO-FC, and I/IIKO-FC or WT-DC provided 100, 66.7, 38.5, 7.7, and 0% protection, respectively, against MC38 tumor challenge (Fig. 4B). The findings were consistent with the CTL activity against MC38/MUC1 and MC38 targets (Fig. 4, C and D). The results indicate that both MHC class I and II Ag presentation contributes to antitumor immunity induced by DC-tumor fusion cells. However, antitumor immunity was more compromised when mice were immunized with fusion cells deficient in MHC class II than with fusion cells deficient in MHC class I molecules.

MHC class I and II Ag presentation required for maximal antitumor immunity to eliminate established tumor

To assess whether immunization with fusion cells can eliminate established tumor, MUC1.Tg mice were injected i.v. with MC38/MUC1 tumor cells and then treated with WTDC-FC, IKO-FC, IIKO-FC, and I/IIKO-FC or WT-DC on days 2 and 8. Treatment with WTDC-FC or IKO-FC rendered 100 and 90% protection, respectively, of MUC1 tumor cells, whereas treatment with IIKO-FC or I/IIKO-FC resulted in 0% protection (Fig. 4C). The findings were consistent with the CTL activity against MC38/MUC1 and MC38 targets (Fig. 4, C and D). The results indicate that both MHC class I and II Ag presentation contributes to antitumor immunity induced by DC-tumor fusion cells. However, antitumor immunity was more compromised when mice were immunized with fusion cells deficient in MHC class II than with fusion cells deficient in MHC class I molecules.

FIGURE 3. Induction of Ag-specific CTL by immunization with DC-tumor fusion cells. A, MUC1.Tg mice ($n = 5$/group) were immunized twice with $5 \times 10^5$ WTDC-FC ( ), IKO-FC ( ), IKO-FC ( ), and I/IIKO-FC ( ) in posterior flank near base of tail. MUC1.Tg mice injected with $5 \times 10^5$ irradiated MC38/MUC1 ( ) were used as control. Splenocytes were collected on day 7 after second immunization and incubated at indicated E:T ratio with $^{51}$Cr-labeled MC38/MUC1, MC38, B16/MUC1, B16, and MCF-7 target cells. CTL activity was determined by $^{51}$Cr release assay. B, Results of CTL assay in repeat experiment.
had tumor growth in the lung, although fewer tumor nodules were found in mice treated with I/IIKO-FC than those treated with DC alone. To assess the CTL status of mice immunized with various types of DC-tumor fusion cells, splenocytes from the immunized mice were isolated and the standard 51Cr release assay was performed. CTL activities against MC38/MUC1 and, to a lesser extent, MC38 or B16/MUC1 were observed in mice immunized with WT-DC (n = 8) and challenged with MC38/MUC1 tumor cells. Sensitivity of CTL was maximal in mice immunized with WT-DC (n = 8), compared to those immunized with IIKO-FC or I/IIKO-FC (Fig. 5C). In contrast, minimal CTL induction occurred in mice immunized with I/IIKO-FC and challenged with MC38/MUC1 and MC38 tumor cells. Similar results were obtained in repeat experiment.

MHC class II-expressing vaccine provided protection against MHC class I- or II-positive tumor challenge

The previous results indicate the differential antitumor immunity induced by I/IIKO-FC. Immunization with I/IIKO-FC affected antitumor immunity much more acutely than that with I/IIKO-FC. One explanation for the differential antitumor immunity induced by I/IIKO-FC is that I/IIKO-FC express MHC class I molecules of tumor origin as demonstrated in FACS analysis (Fig. 1, B and C); thus, the deficiency of MHC class I in DC has been compensated for, at least in part, by MHC class I molecules derived from tumor cells. Therefore, the impact of I/IIKO-FC on induction of CTL and antitumor immunity is not so severe as that of I/IIKO-FC. To address this concern, we created DC-tumor fusion cells devoid of MHC class I expression.

DC isolated from MHC class I knockout mice (IKO-DC) express MHC class II, but not MHC class I molecules, whereas B16/MUC1 express MHC class I and II molecules derived from tumor cells whereas B16/MUC1 express MHC class I and II molecules derived from tumor cells. Therefore, the two models were used to examine the impact of MHC molecules on DC-tumor fusion cell vaccination.

For the I/IIKO-FC model, DC isolated from MHC class I knockout mice (IKO-DC) express MHC class II, but not MHC class I molecules, whereas B16/MUC1 express MHC class I and II molecules derived from tumor cells whereas B16/MUC1 express MHC class I and II molecules derived from tumor cells. Therefore, the two models were used to examine the impact of MHC molecules on DC-tumor fusion cell vaccination.
confirmed these observations (Fig. 6C). The data indicate that MHC class II-expressing vaccine can induce antitumor immunity against both MHC class I- and II-positive tumors.

Discussion

In the present study, we created various DC-tumor fusion cells that deliberately targeted the activation of CD4 and/or CD8 T cells. We have demonstrated: 1) the fusion of DC with tumor cells is a versatile approach to create tumor vaccine; 2) DC-tumor fusion vaccine deficient in MHC class II Ag presentation severely affects the downstream involvement of CD8 and CD4 T cells; and 3) maximal antitumor immune responses require both MHC class I and II Ag presentation, although Ag presentation through MHC class II plays a more important role in the antitumor immunity.

MHC class I and II molecules have a critical function in the selection of CD8 and CD4 T cells in the thymus (26, 27). It has been reported that mice with a disrupted β₂m gene express few MHC class I molecules and are virtually devoid of CD8⁺ T cells (16), and that mice with a disrupted Aα₁ gene lack the expression of class I-A molecules on class II-expressing cells and the development of CD4⁺ T cells (17). In addition, MHC class I and II molecules are important for Ag processing and presentation, and subsequent activation of CD8 and CD4 T cells, respectively. Splenocytes from MHC-deficient mice were poor stimulators in MLR (18) and in allogeneic CTL generation (16). DC from MHC class I- or II-deficient mice were able to prime only CD8 or CD4 T cells, respectively (28). These results are in line with our findings that lack of MHC class I and/or II molecules in DC or DC-based vaccines compromises the activation of T cells. We show, however, the differential magnitude of antitumor immunity effects with IKO-FC or IIKO-FC vaccines; whereas immunization with IKO-FC resulted in slightly decreased CTL induction, tumor prevention, and tumor treatment compared with immunization with WTDC-tumor fusion cells, immunization with IKO-FC abolished IFN-γ production of T cells, significantly impaired CTL induction, and severely compromised the immunotherapeutic effect of T cells in the prevention and treatment experiments. These results indicate that MHC class II Ag presentation targeting CD4 T cells is essential for successful elimination of tumor challenge or established tumors.

CD8 T cells are the focus of study in antitumor immunity because most nonhemopoietic tumors are positive for MHC class I, but negative for MHC class II, and CD8 CTL are the predominant tumoricidal effector cells. Therefore, development of vaccine has been directed toward activation and amplification of CD8 T cells. However, there is increasing evidence that CD4 T cells play a broader role in antitumor immunity (29). Unlike CD8 T cells, CD4 T cells contribute to antitumor immunity through diverse mechanisms. It is well documented that CD4 T cells provide help to CD8 T cells by activating APC through CD40-CD40L interaction (30–32) and/or IL-2 production (33). In addition to providing help in the priming phase, CD4 T cells are also needed in the effector

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**Table I. Numbers and percentage of fused and unfused cells in vaccine**

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a Negative cells were not included.

b % Refers to the percentage of cells positive for MUC1 tumor Ag and/or MHC class II or CD86.

c Indicates the cell numbers.

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FIGURE 6. Prevention of MHC class I- or II-positive tumors in mice vaccinated by DC-tumor fusion cells expressing MHC class II, but deficient in class I molecules (IKO/B16-FC). A. Cells were double stained with FITC-conjugated anti-MUC1 mAb and PE-conjugated anti-MHC class I or anti-MHC class II mAb and analyzed by flow cytometry. B, Prevention of B16/Ia⁺/MUC1 and MC38/MUC1 tumors in IKO/B16-FC-vaccinated mice. Groups of MUC1.Tg mice vaccinated twice with IKO/B16-FC (n = 14). One week after second vaccination, mice were challenged with 5 × 10⁶ B16/Ia⁺/MUC1 tumor cells (○) on right flank and MC38/MUC1 tumor cells (□) on left flank. Groups of MUC1.Tg mice immunized with DC alone (△, n = 3) or with irradiated tumor cells (○, n = 3) were used as controls. Tumor size was measured and tumor incidence was determined on day 30 after tumor challenge. C, Splenocytes were isolated from IKO/B16-FC-vaccinated MUC1.Tg mice on day 30, and incubated with ⁵¹Cr-labeled B16/Ia⁺/MUC1 (○), MC38/MUC1 (△), and MC38 (□) at indicated E:T ratios. CTL activity was determined by the ⁵¹Cr release assay. Results were obtained in two separate experiments.
References

We are indebted to Dr. Suzanne Ostrand-Rosenberg from University of Maryland for the gift of B16Fla melanoma cells.

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