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EFFECTS OF CYTOKINES COMBINED WITH HIGH-DOSE GAMMA IRRADIATION ON THE EXPRESSION OF MAJOR HISTOCOMPATIBILITY COMPLEX MOLECULES AND INTERCELLULAR ADHESION MOLECULE-1 IN HUMAN OVARIAN CANCERS

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Tumor cells from 7 freshly isolated human ovarian tumors and 2 continuous human ovarian cancer cell lines were analyzed for their surface expression of MHC class-I, class-II and ICAM-1 surface antigens before and after exposure to γ-irradiation and/or the cytokines TNF-α plus IFN-γ. All 7 fresh tumors expressed high levels of MHC class-I and ICAM-1 antigens, and levels were markedly up-regulated after exposure to TNF-α plus IFN-γ. Similarly, class-II antigens were either induced (3 out of 7 tumors) or significantly up-regulated by TNF-α plus IFN-γ. Exposure to high doses of γ-irradiation also increased the expression of MHC class-I and ICAM-1 antigens, albeit to a modest degree. MHC class-I and ICAM-1 antigen expression was much lower on continuous human ovarian cell lines than on the fresh tumors. Exposure of these cells to TNF-α plus IFN-γ markedly up-regulated antigen expression to levels comparable to those expressed on the freshly isolated tumors. With the established ovarian cell lines, removal of cytokines caused a rapid down-regulation of antigen expression to basal levels within 6 days, while in the fresh tumors a low level of up-regulation was still present at this time. In contrast, exposure to cytokines followed by high-dose γ-irradiation resulted in a highly significant and long-lasting expression of each surface antigen which was either up-regulated or induced by the cytokines. These data indicate that the combination of these modalities may be beneficial in generating optimal antigen expression for use of tumor cells in vaccine studies.
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A variety of cell-surface molecules have been shown to be involved in the recognition of antigenic cells by the immune system. Major histocompatibility antigens (MHC) and intercellular adhesion molecules play a key role in these cellular interactions. Class-I molecules are expressed on virtually all nucleated cells of the body (Daar *et al.,* 1984) with the exception of some poorly differentiated tumors (Callagen, 1985), while class-II molecules have a more limited distribution (Shackelford *et al.,* 1982). For a specific immune response to ensure, T cells must recognize a target peptide in the context of an MHC molecule. However, accessory molecules such as ICAM-1, which bind to leukocyte-function-associated antigen-1 (LFA-1) (Mart, 1987) and which are expressed on all lymphoid cells, contribute to cellular contact and further contribute to lymphocyte activation (Pircher *et al.,* 1986). Indeed, the presence of antigen-independent contacts has been shown to be particularly important if the avidity between the T-cell receptor an its target antigen on the cell surface is weak (Marianski *et al.,* 1988). In experimental models, the differential expression of class-I antigens (Festenstein, 1987; Vanky *et al.,* 1989), class-II antigens (Pfizenmaier *et al.,* 1985) and ICAM-1 (Vanky *et al.,* 1990) greatly influences the immunogenicity of tumor cells. Moreover, clinical correlation between MHC antigen expression, metastatic potential (Lopez-Nevot *et al.,* 1986) and lymphoid infiltration (Kabawat *et al.,* 1983) has been reported for several tumor types.

Human interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) are potent inducers of MHC and ICAM-1 antigen expression on solid tumors (Carrel *et al.,* 1985) as well as on

non-malignant cells (Collins *et al.,* 1986). There is also ample evidence that IFN-γ can increase the processing and presentation of antigenic peptides by enhancing proteolysis via the proteosome, by increasing the transport of these antigenic peptide fragments into the endoplasmic reticulum, by enhancing the binding of these molecules to an intermediate molecule such as gp96, and by increasing the expression of β_2 microglobulin (Restifo *et al.,* 1994).

Recent advances in molecular genetics, immunology and cancer-cell biology over the last few years have aroused new enthusiasm for vaccine approaches to cancer. In order to induce a specific immune response, tumor vaccines should provide strong activating signals and also express persistently high levels of antigenic molecules. At the same time, when using vaccines derived from single-cell suspensions of allogeneic and/or autologous tumor-cell lines, it is necessary to inhibit cell replication in order to prevent possible tumor formation at the immunization site. High-dose γ-irradiation is one of the most commonly used methods of blocking cell division.

In this study, we examined the effects of cytokine exposure as well as high doses of irradiation on the surface expression of MHC and ICAM-1 molecules in 2 established ovarian carcinoma cell lines and in several freshly isolated tumor-cell suspensions of ovarian origin. We found that irradiation given immediately after a brief exposure to TNF-α plus IFN-γ induces a highly significant and long-lasting expression of each surface antigen which is either up-regulated or induced by the cytokines. Moreover, the combination of the 2 procedures appeared to have an additive effect on the expression of these surface molecules. These findings provide new information for use in the future development of tumor vaccines.

MATERIAL AND METHODS

Fresh tumors and tumor-cell lines

Single-cell suspensions were prepared from 7 ovarian adenocarcinomas of primary or metastatic origin obtained at the time of surgery. Human serous papillary ovarian carcinoma cell lines UCI-101 and UCI-107 were kindly provided by Dr. A. Manetta, University of California, Irvine (UCI). UCI-101 and

Abbreviations: PPC, percentage of positive cells; MFI, mean channel fluorescence intensity; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; MHC, major histocompatibility complex; ICAM-1, intercellular adhesion molecule-1; MAb, monoclonal antibody.

UCI-107 cell lines were maintained at 37°C, 5% CO_2 in complete medium (CM) containing RPMI 1640 (GIBCO Life Technologies, Grand Island, NY) and 10% fetal bovine serum (FBS, Gemini, Calabasas, CA), while fresh single-cell suspensions and short-term cultures were maintained at 37°C, 5% CO_2 in complete medium (CM) containing RPMI 1640, 5% FBS (Gemini). All the fresh tumors were of serous papillary histologic origin. OVA-1, OVA-9, OVA-12 and UCI-107 were obtained from patients who had not been previously treated with chemotherapy, while OVA-6, OVA-7, OVA 13, OVA 14 and UCI-101 were obtained from patients treated with different chemotherapy regimens. Briefly, single-cell suspensions were obtained by processing solid tumor samples under sterile conditions at room temperature. Viable tumor tissue was mechanically minced in RPMI 1640 to portions no larger than 1–3 mm^3 and washed twice with RPMI 1640. The portions of minced tumor were then placed into 250-ml trypsinizing flasks containing 30 ml of enzyme solution [0.14% collagenase Type I (Sigma, St. Louis, MO) and 0.01% DNase (Sigma, 2000 KU/mg)] in RPMI 1640, and incubated on a magnetic stirring apparatus overnight at 4°C. Enzymatically dissociated tumor was then filtered through 150-mm nylon mesh to give a single-cell suspension. The resultant cell suspension was then washed twice in RPMI 1640. Experiments were performed only with suspensions which had at least 90% viability as determined by trypan-blue exclusion. The percentage of tumor cells was determined by cytokeratin expression using immunohistochemical techniques. In this regard, all the fresh tumor samples evaluated contained > 99% tumor cells.

IFN-γ and TNF-α preincubation

Single-cell suspensions of freshly isolated ovarian tumors and the 2 established cell lines were incubated in CM or in CM containing a combination of IFN-γ and TNF-α for 3 days (500 units/ml each) and subsequently evaluated for antigen expression by FACS analysis. Recombinant human IFN-γ (specific activity; 2.5 × 10^7 U/mg) was purchased from Genzyme (Cambridge, MA) and recombinant human TNF-α (specific activity; 1.0 × 10^7 U/mg) was obtained from Genentech (S. San Francisco, CA).

IFN-γ and TNF-α preincubation followed by high-dose γ-irradiation

Fresh ovarian tumors that could be grown as short-term cultures (OVA-6, OVA-7, OVA-9, OVA-12, OVA-13, OVA-14) and the 2 established cell lines were irradiated with a total dose of 10,000 rads with or without pre-incubation with IFN-γ and TNF-α for 3 days (500 units/ml each). One portion of the pre-incubated cells from 2 short-term cultures (OVA-13, OVA-14) and UCI-101 and UCI-107 cell lines was seeded in CM without cytokines just before irradiation. This allowed us to follow the natural course of surface antigen expression with time but without the influence of irradiation. The remaining portion of the same cell cultures was irradiated in 15-ml conical tubes in CM at room temperature with gamma rays (Cesium 137) at a dose rate of 200 rads/min. Immediately after irradiation, cells were seeded into T 75 tissue-culture flasks (Corning, Corning, NY) in CM devoid of cytokines and cultured at 37°C in a 5% CO_2 atmosphere. Spent medium was changed every other day. Irradiated cells preincubated with cytokines, and irradiated control cells were harvested at days 2 and 6 for FACS analysis. Cells from OVA-13, OVA-14, UCI-101 and UCI-107 cell lines pre-incubated with cytokines, not irradiated but put into normal medium just after the completion of pre-incubation (72 hr) were evaluated by FACS at the same time. Cell viability after irradiation was evaluated using trypan-blue dye exclusion. No viable cells were identified after 2 weeks of culture following 10,000 rads of irradiation.

Indirect immunofluorescence and flow cytometry

Cells obtained from single-cell suspensions or after short-term cultures, as well as the established ovarian cell lines, were harvested with 0.25% Trypsin in HBBS (GIBCO), and washed once in CM. Cell suspensions were counted and distributed into 12 × 75-mm tubes at 5 × 10^5 cells/tube. Mouse MAbs [anti-HLA class I (MAb W6/32; Accurate Chemical and Scientific Corp., Westbury, NY); anti-HLA class II (MAb CR3-43; Accurate Chemical); anti-ICAM-1 (MAb LB-2; Becton Dickinson, Mountain View, CA)] were diluted in cold assay buffer (PBS, pH 7.2, supplemented with 0.1% FCS) and added in a 50-ml volume. A mouse IgG preparation (MAb IgG_{2a}; Becton Dickinson) was used as negative control. The fluorescence induction index was determined as the ratio between the mean fluorescence intensity of experimental cells compared to the mean fluorescence intensity of untreated control cells:

induction index

$$= \frac{\text{experimental mean channel fluorescence intensity}}{\text{control mean channel fluorescence intensity}}$$

Statistical analysis

Significance analysis was performed using a paired Student's t test. Only p values < 0.05 were considered significant.

RESULTS

Expression of surface antigens on freshly isolated ovarian tumors and UCI-101 and UCI-107 continuous cell lines

Flow cytometric analysis of MHC class-I and -II antigens as well as ICAM-1 antigen was performed on 7 freshly isolated ovarian tumors and 2 continuous cell lines. The results shown in Table I indicate that MHC class-I antigens were expressed at high levels by all 7 of the freshly isolated ovarian tumors [mean channel fluorescence intensity (MFI) ranged from 855 to 2658]. Class-I antigens were also expressed on the 2 established cell lines, although at a much lower level (MFI evaluated in 3 different experiments ranged from 78 to 211 for UCI-101 and from 155 to 358 for UCI-107). Class-II antigens were expressed at low levels in 4 out of 7 freshly isolated ovarian tumors and then only in a subset of cells, while they were not detected at all on the continuous ovarian cell lines. ICAM-1 was expressed in all the cells evaluated with the exception of UCI-107.

Effect of IFN-γ plus TNF-α on surface antigen expression

The effect of IFN-γ plus TNF-α on the expression of MHC and ICAM-1 antigens on freshly isolated and continuous ovarian tumors was determined by FACS analysis. In all of the ovarian carcinoma cells studied, IFN-γ plus TNF-α markedly up-regulated the level of MHC class-I antigen expression (Table I). The increase in MHC class-I expression by the freshly isolated ovarian tumors ranged from 2.28- to 4.27-fold (induction index), while the increase in expression by UCI-107 and UCI-101 ranged from 11.1 to 29.9 and from 9.1 to 20.5, respectively. IFN-γ plus TNF-α up-regulated the expression of MHC class-II antigens on all freshly isolated ovarian tumors as well as the UCI-107 cell line. In particular, for those tumors which did not express MHC class-II antigens prior to cytokine exposure, IFN-γ plus TNF-α induced its expression, albeit at low levels. In all of the ovarian carcinoma preparations studied, IFN-γ and TNF-α either induced (UCI-107) or up-regulated the expression of ICAM-1 antigens. In this regard, the induction index ranged from 1.64 to 19.2 fold (Table I).

Persistence of surface antigen expression after cytokine withdrawal

The time course of MHC and ICAM-1 surface antigen expression following withdrawal of TNF-α plus IFN-γ was

TABLE I – EXPRESSION OF MHC ANTIGENS AND ICAM-1 ON FRESH OVARIAN TUMORS AND CONTINUOUS OVARIAN CELL LINES, BEFORE AND AFTER EXPOSURE TO TNF-α PLUS IFN-γ

Tumors	Fluorescence	MHC-I			MHC-II			ICAM-1		
		Untreated	TNFα + IFNγ	Ii^1	Untreated	TNFα + IFNγ	Ii	Untreated	TNFα + IFNγ	Ii
OVA-1	PPC	95	96	1.01	46	83	1.80	95	94	0.99
	MFI	855	2157	2.52	54	205	3.79	226	794	3.51
OVA-6	PPC	98	99	1.01	16	79	4.93	92	96	1.04
	MFI	1967	5573	2.83	17	171	10	795	1900	2.38
OVA-7	PPC	84	99	1.17	36	83	2.30	90	91	1.01
	MFI	860	1962	2.28	26	418	16	241	590	2.44
OVA-9	PPC	90	86	0.95	N.D.	41	–	78	84	1.07
	MFI	1278	4824	3.77	N.D.	31	–	111	2140	19.2
OVA-12	PPC	99	99	1.0	N.D.	89	–	97	99	1.02
	MFI	2658	6892	2.59	N.D.	209	–	714	5859	8.20
OVA-13	PPC	68	85	1.25	21	52	2.47	63	93	1.47
	MFI	1096	4685	4.27	144	257	1.78	825	4565	5.53
OVA-14	PPC	97	99	1.02	N.D.	82	–	96	99	1.03
	MFI	1158	4560	3.95	N.D.	84	–	1616	2652	1.64
UCI-101	PPC	90	92	1.02	N.D.	N.D.	–	89	95	1.07
	MFI	78	1601	20.5	N.D.	N.D.	–	96	306	3.19
UCI-107	PPC	96	99	1.03	N.D.	21	–	N.D.	22	–
	MFI	155	3121	20.1	N.D.	19	–	N.D.	58	–

Tumor cells were evaluated for levels of surface antigen expression by flow cytometry before and after exposure to TNF-α plus IFN-γ. Data from one experiment are shown. PPC: percentage positive cels, MFI: mean channel fluorescence intensity. The *p*-values for MIF of cells incubated with cytokines *vs.* controls for MHC I, MHC II and ICAM-1 were 0.0001, 0.05 and 0.03, respectively. N.D., not detectable. –, Not done. Ii, induction index.

TABLE II – PERSISTENCE OF MHC-I AND ICAM-1 ANTIGEN EXPRESSION AFTER CYTOKINE REMOVAL

Tumors	MHC-I Days				ICAM-1 Days			
	−3	0	2	6	−3	0	2	6
UCI-101	1	20.5	6.4*	0.7†	1	3.2	1.1†	0.5†
ICU-107	1	20.1	6.8*	0.6†	–	–	–	–
OVA-13	1	4.3	3.4**	1.8***	1	3.2	1.9**	1.0†
OVA-14	1	4.0	2.5**	2.3***	1	4.1	1.7**	0.8†

Tumor cells were evaluated for levels of surface antigen expression by flow cytometry prior to exposure to TNF-α plus IFN-γ (day-3), immediately after exposure (day 0) or after an additional 2 or 6 days in culture in the absence of cytokines. Values expressed represent induction indexes. Data from one representative experiment are shown.–*p* ≤ 0.001 *vs.* untreated controls; **p* ≤ 0.02 *vs.* untreated controls; ***p* ≤ 0.05 *vs.* untreated controls; †, *p* not significant; –, not done.

studied in 2 freshly isolated ovarian tumors (OVA-13, OVA-14) and the 2 continuous ovarian cell lines. The results of these studies are shown in Table II. For the 2 continuous cell lines, a significant up-regulation of MHC class-I molecules was still present 2 days after removal of the cytokines. However, by 6 days after cytokine withdrawal, no significant difference was noted between treated and untreated cells. When MHC class I and ICAM-1 were evaluated in freshly isolated OVA-13 and OVA-14 tumors, a slower return to the basal (*i.e.* untreated) level of surface antigen expression was noted. Indeed, MHC class-I molecules were still significantly up-regulated after 6 days following cytokine withdrawal (*p* ≤ 0.05).

Expression of surface molecules after high-dose γ-irradiation

Cell-surface antigen expression on 2 fresh ovarian tumors (OVA-13, OVA-14) and the 2 established cell lines was evaluated 2 and 6 days after irradiation and the results were compared to the level of expression of unirradiated control cells. As shown in Table III, for the freshly isolated tumors, irradiation caused a modest but not significant up-regulation of both MHC class-I and ICAM-1 surface antigens which remained at this level for up to 6 days. In contrast, for the continuous cell lines, irradiation induced a marked increase in the expression of class-I and ICAM-1 antigens which persisted for at least 2 days. The marked sensitivity to high-dose irradiation of the established lines prevented the evaluation of

TABLE III – EFFECTS OF IRRADIATION ON MHC-1 AND ICAM-1 ANTIGEN EXPRESSION ON FRESH OVARIAN TUMORS AND CONTINUOUS OVARIAN CELL LINES

Tumors	MHC-I days			ICAM-1 days		
	0	2	6	0	2	6
UCI-101	1	2.7*	–	1	1.3*	–
UCI-107	1	3.9*	–	N.D.	N.D.	–
OVA-13	1	1.1†	1.2†	1	1.1†	1.0†
OVA-14	1	1.1†	1.1†	1	1.3†	1.0†

Tumor cells were evaluated for levels of surface antigen expression by flow cytometry prior (day 0) or after 2 or 6 days after high-dose gamma irradiation. Values expressed represent induction indexes. Data from one representative experiment are shown.–*p* ≤ 0.05; †*p* not significant. N.D., not detectable. –, Not done.

antigen expression after 6 days. It is interesting that irradiation did not induce neoexpression of antigens previously not present on these cells, such as MHC class II. It is also worth noting that, while irradiation could cause an increased expression of all antigens tested expressed by the cells, the levels reached were much lower than those attained by exposure to IFN-γ and plus TNF-α.

Expression of surface molecules after irradiation with or without previous exposure to IFN-γ plus TNF-α

Cell-surface antigens were evaluated 2 and 6 days after irradiation in 6 freshly isolated ovarian tumors (OVA-6,

TABLE IV – EXPRESSION OF MHC-I AND ICAM-1 ANTIGENS ON IRRADIATED ESTABLISHED CELL LINES WITH AND WITHOUT PRIOR CYTOKINE EXPOSURE

Tumors	MHC-1 day 2					ICAM-1 day 2				
	Control	Irradiation only	Ii[1]	Cytokines + irradiation	Ii	Control	Irradiation only	Ii	Cytokines + irradiation	Ii
UCI-101	78	210	2.7	1703	21.8	96	126	1.3	257	2.7
UCI-107	155	611	3.9	4585	29.5	—	—	—	46	—

Tumor cells were evaluated by FACS analysis for levels of surface antigen expression 2 days after irradiation with or without prior cytokine exposure. Data are expressed as MFI and as induction indexes. Data from one representative experiment are shown. The *p*-values for MHC class I and ICAM-1 after 2 days were 0.02 and 0.05, respectively.–Ii, Induction indexes.

OVA-7, OVA-9, OVA-12, OVA-13, OVA-14) and after 2 days in the established cell lines with or without prior exposure to IFN-γ plus TNF-α. In all the tumor cells studied, prior exposure to cytokines followed by irradiation induced a highly significant and long-lasting expression of all the surface antigens either up-regulated or induced by the cytokines (Table IV and Fig. 1). Indeed, in the freshly isolated tumors, the levels of antigen expression following cytokine exposure and irradiation remained significantly elevated for up to 6 days. This was in marked contrast to the effects of cytokine exposure only, in which surface antigen expression rapidly and consistently decreased after treatment. In addition, when the level of antigen up-regulation reached with cytokine exposure at day 0 and day 2 was compared to the level of antigen expression after the combination of the 2 procedures (day 2), an additive and persistent increase in antigen expression was noted both for the freshly isolated tumors and for the continuous cell lines (Fig. 2).

DISCUSSION

This study investigated the expression of MHC and ICAM-1 surface antigens on freshly isolated and continuous cell lines of human ovarian epithelial tumors. The effects of high-dose γ-irradiation, the exposure to cytokines TNF-α plus IFN-γ and the combination of the 2 procedures on the expression of these antigens were carefully studied. The results indicate that our series of freshly isolated ovarian epithelial tumors express high levels of MHC class-I and ICAM-1 antigens compared to the 2 continuous cell lines, and that 4 out of these 7 tumors also expressed detectable levels of MHC class-II antigens. Following exposure to TNF-α plus IFN-γ, all tumors studied rapidly up-regulated their levels of expression of MHC class I and ICAM-1. Furthermore, in those tumors which already expressed MHC class-II antigens (4 out of 7), cytokine exposure also markedly increased its surface expression. In those tumors which did not express MHC class-II antigens, exposure to cytokines induced their expression in all cases. In the continuous ovarian cell lines, MHC and ICAM-1 antigens were expressed at a much lower level than in the freshly isolated tumors, but exposure to cytokines also markedly up-regulated their level of expression to values comparable to those seen for the fresh tumors. It is worth noting that the striking difference in the up-regulation of surface molecules seen in the 2 groups (induction index for MHC I from 2.28 to 4.27 in the fresh tumors, compared to an induction index of 9.1 to 29.9 in UCI-101 and UCI-107 cell lines) appears to be due to different levels of expression in the resting basal state and shows that cells which express low levels of these surface molecules are capable of up-regulating them to very high levels after exposure to the appropriate cytokines. It is noteworthy that exposure to TNF-α plus IFN-γ did not cause any observable toxic effects to any of the freshly isolated tumor samples but appeared to decrease the growth rate of the established ovarian cell lines.
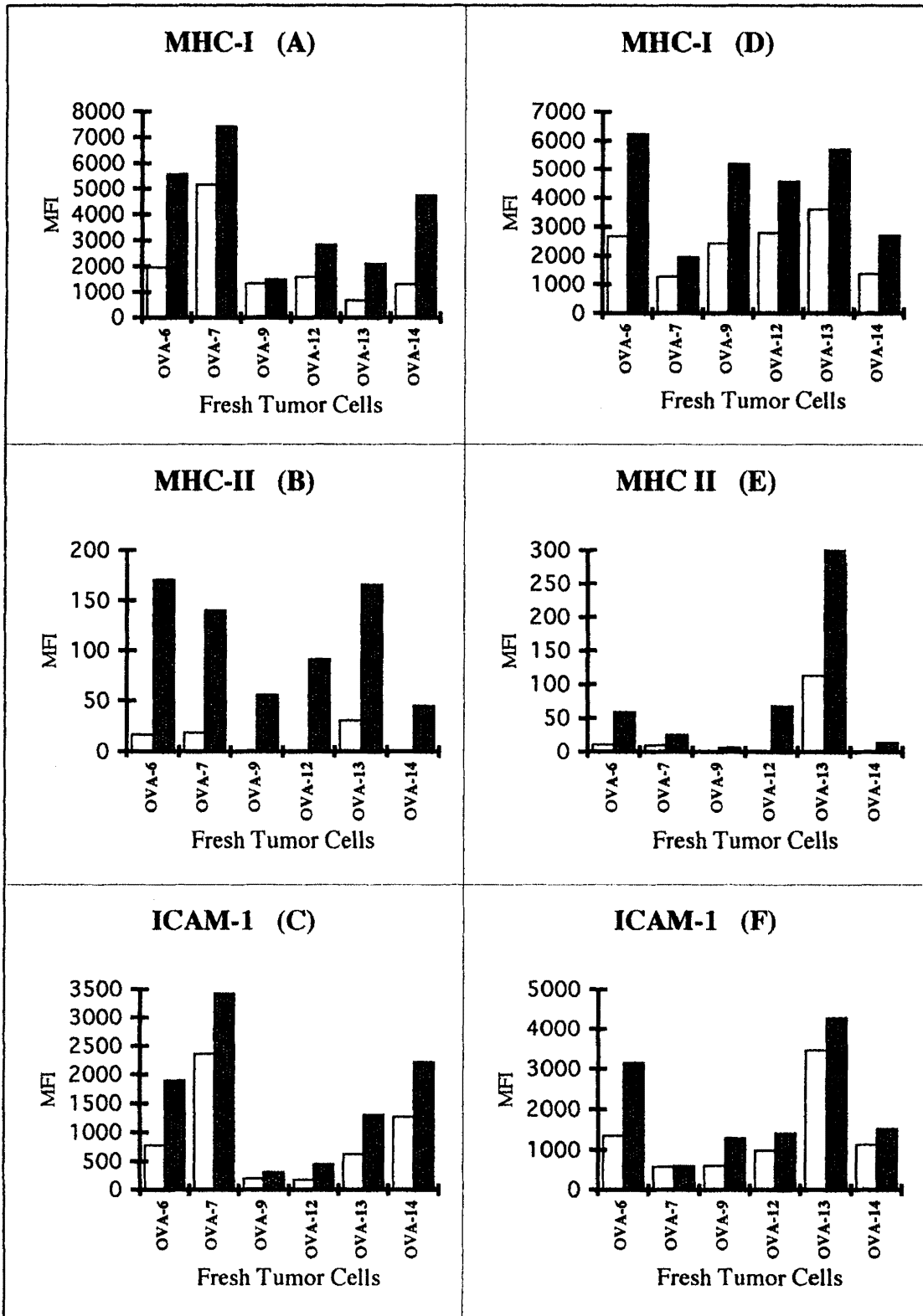
The effect of γ-irradiation on surface antigen expression was also investigated. Our results indicate that, in all tumors evaluated, an increase in the expression of MHC class-I and

ICAM-1 antigens consistently occurred, although this increase was statistically significant only in the 2 continuous cell lines and was only modest when compared to the up-regulation induced by the cytokines. Our previous studies showed that irradiation up-regulated other antigens in addition to MHC and ICAM-1 (*i.e.* Her-2/Neu) and that this was dose-dependent, higher expression being associated with higher doses of irradiation (data not shown). In the present study, in agreement with other reports (Hauser *et al.*, 1993, Hareyama *et al.*, 1991), we were able to confirm that an increase in expression of surface antigens is a general feature induced by high doses of γ-irradiation. However, such up-regulation, unlike that caused by TNFα plus IFNγ, did not induce expression of antigens not previously expressed in the cell lines prior to irradiation.

The increased level of antigen expression observed following exposure to TNF-α plus IFN-γ was not persistent and dropped rapidly after removal of the cytokines. However, a somewhat slower return to the basal surface expression was noted in the fresh tumors.

Our study design differed from that of previous reports evaluating the effects of irradiation on antigen expression (Hauser *et al.*, 1993; Hareyama *et al.*, 1991) inasmuch as the cells were preincubated with cytokines prior to irradiation. We found that, following exposure to cytokines, irradiation caused a highly significant and long-lasting expression of all the surface antigen, either up-regulated or induced by the cytokines. Moreover, a further additive effect was noted upon increasing the expression of the surface molecules, either up-regulated or induced by the cytokines. Finally, the combination of such procedures was clearly shown to be superior to either modality alone.

The importance of these findings is underscored by previous reports on the influence of surface antigen expression and immune recognition. Several studies on human tumor cells of different origins exposed to TNF-α plus IFN-γ have shown that the induction of cytotoxic effector cells requires a higher level of antigen expression than cytotoxic interaction (Vanky *et al.*, 1989; Anichini *et al.*, 1993). In fact, tumors not previously considered to be immunogenic but so induced to express higher levels of MHC antigens appear to recruit high-avidity T cells, which can then recognize target cells which express virtually undetectable levels of MHC antigens (Vanky *et al.*, 1989). Moreover, in studies on melanoma (Anichini *et al.*, 1993), the expression of ICAM-1 in different melanoma clones, correlated with the susceptibility of the tumor cells to lysis by specific and non-specific T-cell clones and, more importantly, up-regulation of ICAM-1, by treatment with IFN-γ in low-expressor clones, could boost tumor lysability only by specific, TCR-dependent and HLA-restricted effector cells. In such studies, the relative surface expression of ICAM-1 was a key factor for the outcome of the interaction with T cells and only those clones that expressed these molecules at levels high enough for an efficient interaction with antigen-specific T cells were able to trigger lysis.

FIGURE 1 – Effects of irradiation *versus* cytokine plus irradiation after 2 and 6 days on the expression of MHC class-I, class-II and ICAM-1 surface antigens on 6 freshly isolated ovarian tumors. Data from one representative experiment are shown. *a, b, c,* Results after 2 days. *d, e, f,* Results after 6 days. Open columns: irradiation only. Hatched columns: cytokine exposure followed by irradiation. The *p*-values after 2 and 6 days were 0.006 and 0.002, for MHC class I, 0.001 and 0.05 for class II and 0.004 and 0.04 for ICAM-1, respectively.
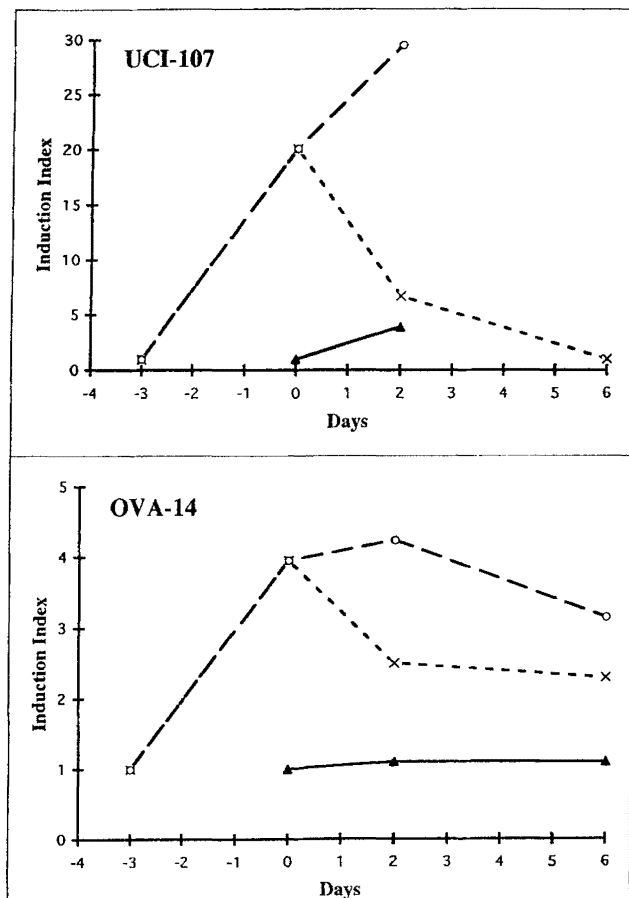
FIGURE 2 – Effects of cytokine exposure, irradiation, and the 2 procedures combined on the expression of MHC class-I in UCI-107 and OVA-14. Tumor cells were evaluated by flow cytometry prior to exposure to TNF-α plus IFN-γ (day − 3), immediately after exposure to cytokines (day 0) or after an additional 2 and 6 days following cytokine withdrawal and with or without subsequent irradiation. Data from one representative experiment are shown. (▲) Irradiation only; (x) cytokine exposure only; (○) cytokine exposure followed by irradiation. The data shown are representative of one experiment for each tumor shown. However, additional experiments performed with UCI-101 and OVA-13 showed similar results.

Other studies with human tumor cells deficient in antigen processing (Restifo et al., 1994) have also shown a greatly enhanced antigen-presenting capacity after exposure to IFN-γ, suggesting that even the molecules that are putatively involved in antigen processing can be up-regulated after such treatment.

The importance of the direct expression of MHC class-II antigens on tumor cells has been highlighted in recent studies of transduction of class-II genes in murine tumor models (Clements et al., 1992). Such studies have shown that the expression of class-II molecules can make such tumor cells function as antigen-presenting cells provided that the invariant chain (Ii) is not expressed (Clements et al., 1992). Indeed, because most non-lymphoid and non-myeloid-derived murine tumor cells do not express Ii (Koch and Harris, 1984) and the expression of MHC class-II molecules can occur in human cells in the absence of Ii (Sekaly et al., 1986), the possibility of increasing or of inducing expression of such molecules after cytokine treatment could also augment the possibility of direct activation of CD4 helper-inducer T cells. Finally, in a prospective randomized clinical trial of colon carcinoma, Hanna et al. (1993) demonstrated that immunization with autologous tumor cells mixed with BCG led to a significant increase in disease-free survival only in those patients whose tumor cells expressed high levels of MHC class-II and ICAM-1 surface antigens.

Until common tumor antigens are identified at the genetic level, and the prevalence and biorelevance of these antigens are assessed, whole tumor cells remain the most reliable source of specific tumor antigens for cancer immunotherapy. The possibility of increasing or inducing de novo expression of MHC molecules and ICAM-1 or other important surface antigens in tumor cells after exposure to TNF-α plus IFN-γ has already been recognized as an efficient method of increasing in vitro induction of T-cell responses against these tumors (Vanky et al., 1989, Pfizenmaier et al., 1985). However, one of the problems to consider when such up-regulated cells are used as tumor vaccines is the relatively short half-life of the up-regulation following removal of the cytokines. Moreover, when using vaccines derived from single-cell suspensions or from allogeneic-autologous established tumor-cell lines, it is necessary to inhibit cell replication in order to prevent tumor formation at the immunization site. Such procedures should not compromise the immunogenicity of the vaccine; rather, they should maintain or even enhance the intrinsic immunogenic potential. High doses of γ-irradiation represent one of the most commonly used methods of inhibiting cell replication for human tumor immunotherapy. Taken together, the observations reported here support the use of a combination of such procedures when preparing tumor cells for use as tumor vaccines. These important findings provide new and additional strategies for active specific immunization in patients with cancer.

REFERENCES

ANICHINI, A., MORTARINI, R., ALBERTI, S., MONTOVANI, A. and PARMIANI, G., T-cell-receptor engagment and tumor ICAM-1 up-regulation are required to by-pass low susceptibility of melanoma cells to autologous CTL-mediated lysis. Int. J. Cancer, 53, 994–1001 (1993).

CALLAGEN, G.N., Products of the major histocompatibility complex on tumor cells. In: S. Sell and R.A. Reisfeld (eds.), Monoclonal antibody and cancer, pp. 97–121, Humana Press, Clifton, NJ (1985).

CARREL, S., SCHMIDT-KESSEN, A. and GIUFFRÉ, L., Recombinant IFN-g can induce the expression of HLA-ABC and tumor associated antigens. Europ. J. Immunol., 15, 118–123 (1985).

CLEMENTS, V.K., BASKAR, S., ARMSTRONG, T.D. and OSTRAND-

ROSENBERG, S., Invariant chain alters the malignant phenotype of MHC Class II⁺ tumor cells. J. Immunol., 149, 2391–2396 (1992).

COLLINS, T., LAPIERRE, L.A., FIERS, W., STROMINGER, J.L. and PROBER, J.S., Recombinant tumor necrosis factor increase in RNA levels and surface expression of HLA-A, B antigens in vascular endothelial cells and dermal fibroblast in vitro. Proc. nat. Acad. Sci. (Wash.), 83, 446–450 (1986).

DAAR, A.S., FUGGLE, S.V., FABRE, J.V., TING, A. and MORRIS, P.J., The detailed distribution of HLA-ABC antigens in normal human organs. Transplantation, 38, 287–292 (1984).

FESTENSTEIN, H., The biological consequences of altered MHC expression on tumors. Brit. med. Bull., 43, 217–227 (1987).

HANNA, M.G., JR., RANSOM, J.H., POMATO, N., PETERS, L., BLOEMENA, E., VERMORKEN, J.B. and HOOVER, H.C., JR., Active specific immunotherapy of human colorectal carcinoma with an autologous tumor cell/bacillus Calmette-Guérin vaccine. *Ann. N.Y. Acad. Sci.,* **690,** 135–146 (1993).

HAREYAMA, M., IMAI, K., KUBO, K., TAKAHASHI, H., KOSHIBA, H., HINODA, Y., SHIDOU, M., OOUCHI, A., YACHI, A. and MORITA, K., Effect of radiation on the expression of carcinoembryonic antigen of human gastric adenocarcinoma cells. *Cancer,* **67,** 2269–2274 (1991).

HAUSER, S.H., CALORINI, L., WAZER, D.E. and GATTONI-CELLI, S., Radiation-enhanced expression of major histocompatibility complex class-I antigens H-2D^b in B16 melanoma cells. *Cancer Res.,* **53,** 1952–1955 (1993).

KABAWAT, S.E., BAST, R.C., JR., WELCH, W.R., KNAPP, R.C. and BAHN, A.K., Expression of major histocompatibility antigens and nature of inflammatory cellular infiltrate in ovarian neoplasms. *Int. J. Cancer,* **32,** 547–554 (1983).

KOCH, N. and HARRIS, A.W., Differential expression of the invariant chain in mouse tumor cells: relationship to B lymphoid development. *J. Immunol.,* **132,** 12–15 (1984).

LOPEZ-NEVOT, M.A., GARCIA, E., PAREJA, E., BONAL, F.J., MARTIN, J., RUIZ-CABELLO, F., SERRANO, S. and GARRIDO, F., Differential expression of HLA antigens in primary and metastatic melanomas. *J. Immunogenet.,* **13,** 219–227 (1986).

MARIANSKI, J.L., PALA, P., CEROTTINI, J.CH. and MACDONALD, H.R., Antigen recognition by H-2 restricted cytolytic lymphocytes: inhibition of cytolysis by anti-CD8 monoclonal antibodies depends upon both concentration and primary sequence of peptide antigen. *Europ. J. Immunol.,* **18,** 1863–1866 (1988).

MART, E., LFA-1 and other accessory molecules functioning in adhesions of T and B lymphocytes. *Hum. Immunol.,* **18,** 3–37 (1987).

PFIZENMAIER, K., BARTSCH, H., SCHEURICH, P., SELIGER, B., UCER, U., VEHMEYER, K. and NAGEL, G.A., Differential gamma-Interferon response of human colon carcinoma cells: inhibition of proliferation and modulation of immunogenicity as independent effects of gamma-Interferon on tumor cell growth. *Cancer Res.,* **45,** 3503–3509 (1985).

PIRCHER, H., GROSCURTH, P., BAUMHUTTER, S., AGUET, M., ZINKER-NAGEL, R.M. and HENGARTENER, H., A monoclonal antibody against altered LFA-1 induces proliferation and lymphokine release of cloned T cells. *Europ. J. Immunol.,* **16,** 172–181 (1986).

RESTIFO, N.P., Antigen presentation by IFN-γ releasing tumor cells. *In:* G. Forni, R. Foa, A. Santoni and L. Frati (eds.), *Cytokine-induced tumor immunogenicity. From exogenous molecules to gene therapy,* pp. 307–327, Academic Press, London (1994).

SEKALY, R.P., TONNELLE, C., STRUBIN, M., MACH, B. and LONG, E.O., Cell surface expression of class II histocompatibility antigens occurs in the absence of the invariant chain. *J. exp. Med.,* **164,** 1490–1504 (1986).

SHACKELFORD, D.A., KAUFMAN, J.F., KORMAN, A.J. and STROMINGER, J.L., HLA-DR antigens: structure, separation of subpopulations, gene cloning and function. *Immunol Rev.,* **66,** 133–187 (1982).

VANKY, F., STUBER, G., ROSTEIN, S. and KLEIN, E., Auto-tumor recognition following *in vitro* induction of MHC antigen expression on solid human tumors: stimulation of lymphocytes and generation of cytotoxicity against the original MHC-antigen negative tumor cells. *Cancer Immunol. Immunother.,* **28,** 17–21 (1989).

VANKY, F., WANG, P., PATARROYO, M. and KLEIN, E., Expression of the adhesion molecule ICAM-1 and the major histocompatibility complex antigens on human tumor cells is required for their interaction with autologous lymphocytes *in vitro. Cancer Immunol. Immunother.,* **31,** 19–27 (1990).