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The Role of Intercellular Adhesion Molecule-1/LFA-1 Interactions in the Generation of Tumor-Specific CD8+ T Cell Responses1

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The activation of naive CD4+ T cells requires both TCR engagement and a second costimulatory signal mediated by the interaction of CD28 with CD80/CD86 expressed on professional APC. However, the situation for naive CD8+ T cells is less clear. Although evidence indicates that induction of CD8+ T cell responses is also dependent on professional APC, the ability of some tumors, which do not express CD80/CD86, to induce CTL suggests that other pathways of costimulation exist for the activation of CD8+ T cells. We examined the ability of tumor cells expressing different levels of a tumor-specific Ag to directly prime CD8+ T cells. We demonstrate that CD8+ T cells are directly activated by tumor cells in a CD80/CD86-dependent manner. In this system, costimulation requires ICAM-1/LFA-1 interaction. This results in the generation of CTL capable of inhibiting tumor growth in vivo, and maintaining long-term survival. The Journal of Immunology, 2005, 174: 3401–3407.

The signaling requirements for naive CD8+ T cell priming are not fully understood, but may involve several factors such as the density of MHC-Ag complexes, the availability of costimulatory molecules, and the presence of CD4+ T cell help (1–3). Current dogma for T cell activation implies that two signals are required (4, 5). Signal 1 is provided by the ligation of the TCR by an MHC-Ag complex. Signal 2 is thought to be mainly provided by ligation of CD28 by the classical costimulatory molecules of the B7 family (CD80 and CD86), expressed predominantly on professional APCs. It has been proposed that signal 1, in the absence of signal 2, induces T cell tolerance (4, 6, 7). As peripheral epithelial tissues, and hence the tumors derived from them, do not constitutively express CD80 or CD86 molecules it is possible that any direct naive T cell/tumor interaction may lead to tolerance. A role for professional APC, capable of expressing co-stimulatory molecules, in capturing and cross-presenting tumor Ag would therefore seem to be a prerequisite for the activation of naive tumor-specific CD8+ T cells. Currently the role and outcome of direct priming of CD8+ T cell responses by tumor cells remains unclear.

Despite the lack of classical costimulation provided by the majority of tumor cells, some reports demonstrate that antitumor CTL may be generated by direct priming by tumor cells (8–10). Although one report demonstrated that this did not require signaling through CD28 (11), the mechanism by which tumor cells were able to provide costimulation was unclear. In general, the requirement for CD28 signaling in the activation of CD8+ T cells is less apparent than for CD4+ T cells. Alternative CD28-independent pathways for T cell priming are therefore of interest when examining mechanisms of direct priming by tumor cells.

In vivo priming of tumor-specific CD8+ T cells has been shown to occur by either cross-presentation of tumor Ag (12–15) or by direct presentation (8–10). Current evidence demonstrates that direct priming requires tumor cells to be present in the lymph node (9, 16). Although for cross-presentation, Ag is carried into the lymph node (16) where it is presented by professional APC that then provide costimulatory signals through CD28-dependent pathways (11). It seems likely however, that both direct and indirect pathways may contribute to T cell priming (10, 11, 16), where they may play roles at different stages of tumor development. Thus, it is possible that Ag available for cross-presentation may precede Ag availability for direct priming. The mechanism by which naive antitumor CD8+ T cells are activated is therefore clearly of interest. Firstly, activation may lead to the induction of tolerance or result in the generation of effector cells. Secondly, direct priming may be associated with the presentation of different epitopes other than those made available by cross-presentation, thereby affecting the repertoire of T cells able to make an antitumor response.

In this study we examine the consequences of direct CD8+ T cell interactions with tumor cells. Murine renal carcinoma (Renca) cells (17) were transfected with the hemagglutinin (HA)3 gene from influenza virus A/PR/8 to generate cell lines expressing high, intermediate, and low levels of HA protein. The use of transgenic clone 4 CD8+ T cells, expressing a high avidity TCR specific for the immunodominant Kb-restricted epitope of HA (518-IYS-TVASSL-526) (18) has enabled us to study the results of naive CD8+ T cell interaction with tumor cells both in vitro and in vivo.

Materials and Methods

Mice

BALB/c mice and BALB/c clone 4 TCR transgenic mice (18) were bred under specific pathogen-free conditions at the University of Bristol animal facility. All experiments were conducted in accordance with U.K. Home Office guidelines.

Transfection and cloning of HA-expressing cell lines

The RCL3 cell line was single cell-cloned from a population of Renca cells (17) (originally obtained from Dr. H. Levitsky, Johns Hopkins University, University Walk, Bristol BS8 1TD, U.K. E-mail address: D.J.Morgan@bristol.ac.uk)
Baltimore, MD) and termed RencaNT. Cells were grown in routine medium (RPMI 1640, 10% v/v FCS, 2 mM glutamine, 50 U/ml penicillin/ streptomycin, 5 × 10⁻⁵ M 2-ME).

For transfection, 2.4 × 10⁶ RencaNT cells were electroporated at 180 V, 975 μF with 20 μg of the pKG10 expression vector (a gift from Dr. K. G. Gould, Imperial College, London, U.K.) for HA (influenza A/PR/8/34). Some 72 h later, cells were passed into routine medium containing 1 mg/ml geneticin G418 and cultured for 2 wk. Resulting colonies were ring cloned and expanded and tested for HA expression. Colonies that stained positively for HA were single cell cloned to produce cell lines expressing different levels of HA; Renca HA<sub>b</sub> high, Renca HA<sub>m</sub> med, Renca HA<sub>low</sub>. These cell lines were maintained in routine medium supplemented with 0.1 mg/ml geneticin (Invitrogen Life Technologies).

**Characterization of cell lines**

Cells were stained for HA expression using 37/38 Abs followed by goat anti-mouse IgG-FITC (Sigma–Aldrich) secondary Ab. Cells were analyzed using a FACScalibur (BD Biosciences) flow cytometer.

For RT-PCR, total RNA was isolated from cell lines using an SV total RNA isolation kit (Promega) and cDNA synthesized using a CDNA synthesis kit (Invitrogen Life Technologies). PCR was conducted for HA and actin using the following primers: 5′-CAATTGGGGGAAATGTAACATTGCCG-3′, 5′-AGCCTTGGGATGAGCCCTCCTC-3′; actin 5′- GTGCACCTGCTACGCA-3′, 5′-TGCGGTACCTCCTGCTGAAA-3′. Cycling conditions were 94°C/5 min, 28 cycles for 94°C/30 s, 61°C/30 s.

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**Cell lines were cultured in routine medium alone, or supplemented with 10 ng/ml recombinant IFN-γ for 48 h, and then stained for cell surface markers using the following mAb (all BD Pharmingen), anti-ICAM-1 FITC, anti-H-2Kd PE, anti-CD11a FITC, anti-CD80 biotin, anti-CD86 biotin, anti-4-1BBL, and OX40L (BD Pharmingen) Abs. Cells were analyzed on a FACScalibur (BD Pharmingen).**

**Coculture assays**

A total of 1 × 10⁶ Renca cells were cultured with 1 × 10⁶ clone 4 CD8<sup>+</sup> T cells (18). Clone 4 T cells were isolated and purified as described earlier. A total of 10⁶ Thy1.1<sup>+</sup> Thy1.2<sup>+</sup> T cells were incubated at 37°C in a humidified incubator with 5% v/v CO₂ for 48 h, and then stained for cell surface markers using the following mAb (all BD Pharmingen), anti-ICAM-1 FITC, anti-H-2Kd PE, anti-CD11a FITC, anti-CD80 biotin, anti-CD86 biotin, anti-4-1BBL, and OX40L (BD Pharmingen) Abs. Cells were analyzed on a FACScalibur (BD Pharmingen).**

**Characterization of RencaHA cell lines**

A single cell clone capable of tumor growth in vivo was established from the Renca cell population (RencaNT) (17). The stable transfection of the RencaNT cell line with an expression vector for the HA gene of influenza virus A/PR/8 yielded three cell lines. These lines were termed Renca HA<sub>b</sub> high, HA<sub>m</sub> med, and HA<sub>low</sub> as determined by the level of HA expression shown by flow cytometric analyses (Fig. 1, A–D) and by RT-PCR (Fig. 1E).

The RencaNT and HA cell lines were further characterized for expression of surface markers known to be involved in the induction of T cell responses (Fig. 2). The cell lines were also cultured in the presence of IFN-γ for 48 h before analyses to determine whether these molecules were likely to be up-regulated during proinflammatory immune responses or following encounter with T cells in culture. Flow cytometric analyses revealed that all of the cell lines expressed MHC class I H-2Kd. None of the cell lines expressed the classical costimulatory molecules CD80 or CD86, nor CD11a (LFA-1), 4-1BBL, or OX40L (BD Pharmingen).

**Results**

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Similarly, HA int cells also acted as targets for clone 4 CTL killing, RencaHA cell lines directly activate naive clone 4 CD8 T cells in an Ag-specific manner. However, all of the cell lines expressed ICAM-1, which was upregulated in the presence of IFN-γ.

The ability of the RencaHA tumor cell lines to act as targets for HA(518-526)-specific CTL was determined using a standard 51Cr release assay. Clone 4 CTL were added to cultures of 51Cr-labeled Renca cell lines to determine the ability of the tumor cells to act as targets. The HA<sup>high</sup> cells were lysed by clone 4 CTL with equal efficiency as when pulsed with HA<sub>518-526</sub> peptide (Fig. 3). Similarly, HA<sup>int</sup> cells also acted as targets for clone 4 CTL killing, however, lysis was less than that seen when these cells were pulsed with HA<sub>518-526</sub> peptide. Lysis of the HA<sup>low</sup> cell line was just above background. These data suggested that the HA-transfected cell lines were expressing H-2K<sup>b</sup> complexes, which were binding HA epitopes, and that susceptibility to lysis by clone 4 CTL was related to the level of HA protein expression on the cell surface.

**RencaHA cell lines directly activate naive clone 4 CD8<sup>+</sup> T cells**

To ascertain whether HA-expressing Renca tumor cells could directly activate naive CD8<sup>+</sup> T cells in vitro, tumor cell lines were cocultured for 48 h with purified naive clone 4 CD8<sup>+</sup> T cells. Clone 4 cells were isolated from the cocultures and analyzed by flow cytometry to determine the level of forward scatter (related to cell size) and expression of the early activation marker CD69 (Fig. 4A).

Clone 4 T cells cultured alone or with RencaNT cells exhibited low forward scatter and did not express CD69, suggesting that these cells were not activated. In contrast, naive clone 4 T cells cultured with the HA-expressing Renca cell lines had high forward scatter and expressed CD69, suggesting that HA expression by these tumor cells resulted in the activation of naive clone 4 CD8<sup>+</sup> T cells. Interestingly, the geometric mean of the forward scatter values and CD69 expression among the clone 4 cells correlated with the level of HA expression on the tumor cell line.

Up-regulation of CD69 expression on clone 4 T cells can occur both in the generation of a productive response that results in effector CTL or during an abortive response associated with tolerance induction (19). However, IFN-γ production has been shown to only occur in the generation of a productive response (19). To determine whether direct activation of naive clone 4 T cells by the RencaHA cell line resulted in the generation of effector cells, coculture supernatants were analyzed for the presence of IFN-γ by ELISA (Fig. 4B). IFN-γ was detected only in supernatants from clone 4 T cells cultured in the presence of HA-expressing Renca cell lines. IFN-γ was not detected in the supernatants from clone 4 cells or tumor cells cultured alone, or clone 4 cells cultured with RencaNT cells. To discount the possibility that clone 4 T cell activation may be due to the presence of contaminating dendritic cells among the clone 4 preparation, clone 4 T cells were cocultured with the HA-expressing Renca cell lines had high forward scatter and did not express CD69, suggesting that HA expression by these tumor cells resulted in the activation of naive clone 4 CD8<sup>+</sup> T cells. Interestingly, the geometric mean of the forward scatter values and CD69 expression among the clone 4 cells correlated with the level of HA expression on the tumor cell line.
with each of the HA-expressing Renca cell lines. The clone 4 T cells cocultured with the RencaHA cell lines were able to lyse HA_{518–526} peptide-pulsed P815 targets (Fig. 5). Target cell lysis was shown to be Ag-specific as unpulsed P815 target cells were not lysed by clone 4 T cells primed by any of the RencaHA cell lines. Control clone 4 cells cocultured with RencaNT cell lines did not lyse HA_{518–526} peptide-pulsed P815 targets.

**Tumor-activated CTLs kill tumor cells in vitro**

To determine whether tumor-activated clone 4 cells were able to lyse tumor targets and the effect of Ag expression levels at both priming and effector phases, activated clone 4 CTL were generated by coculture with each of the Renca HA^{high}, HA^{int}, and HA^{low} cell lines and also HA_{518–526} peptide-pulsed splenocytes as controls. The CTL activity toward ^51Cr-labeled RencaHA cell lines and P815 cells pulsed with and without HA_{518–526} peptide was examined in vitro. The data show that all of the RencaHA cell line cocultures contained H-2K^{d} HA-specific clone 4 effector cells (Fig. 6) as demonstrated by their ability to lyse HA_{518–526} peptide-pulsed but not unpulsed P815 cells (Fig. 6, A–C).

Clone 4 CTL generated from cocultures with Renca HA^{high}, HA^{int}, or HA^{low} cell lines were all able to lyse unpulsed HA^{high} targets with near equal efficiency as when pulsed with HA_{518–526} peptide (Fig. 6, E–G). In contrast, endogenous levels of HA protein on the HA^{int} targets induced lower levels of tumor cell lysis than when pulsed with HA_{518–526} peptide (Fig. 6, I–K). Similarly, endogenous levels of HA protein on the HA^{low} cell line targets induced even lower levels of tumor cell lysis than when pulsed with HA_{518–526} peptide (Fig. 6, M–O). Therefore, the ability of the clone 4 CTL populations to lyse the various HA-expressing tumor targets was not dependent upon the level of HA expression available during priming in the cocultures. However, the level of tumor cell lysis by all populations of clone 4 CTL was dependent upon the level of endogenous HA expression by the target tumor cells.

**Activation of naive clone 4 T cells by RencaHA cells can be blocked by inhibition of the ICAM-1/LFA-1 interaction**

Several studies have determined that the activation of naive CD8^{+} T cells to form effector CTL requires the expression of the classical costimulatory molecules CD80 and CD86 by APC (2). Given that

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**FIGURE 4.** Activation of clone 4 T cells by HA-expressing tumor cell lines. A, Clone 4 T cells that had been cocultured alone or with the tumor cell lines RencaNT, HA^{high}, HA^{int}, or HA^{low} were incubated with anti-CD8, anti-CD69, and H-2K^{d} HA tetramer. CD8^{+} tetramer^{+} clone 4 T cells were examined for activation in terms of cell size (forward scatter) and expression of CD69. The geometric mean for both forward scatter and CD69 expression is given for each histogram. B, Purified clone 4 T cells were cultured with the tumor cell lines ( ), or tumor cells were cultured alone ( ) for 48 h and IFN-γ production determined by ELISA. Data are representative of three separate experiments ± SEM. C, Clone 4 T cells purified by cell sorting on CD8^{+} and Thy1.1 were cultured with the tumor cell lines ( ), or tumor cells were cultured alone ( ) for 48 h and IFN-γ production determined by ELISA.

**FIGURE 5.** Clone 4 T cells primed with HA-expressing tumor cell lines can lyse P815 HA_{518–526} peptide-pulsed targets. Clone 4 T cells were cocultured with the RencaNT, HA^{high}, HA^{int}, or HA^{low}. Their ability to lyse unpulsed P815 ( ) and HA_{518–526} peptide-pulsed P815 targets ( ) was measured using a standard ^51Cr release assay. Data are representative of four experiments.

**FIGURE 6.** Clone 4 T cells primed on HA-expressing tumor cells can kill HA-expressing tumor cells. Clone 4 T cells were cocultured with the HA-expressing tumor cell lines HA^{high}, HA^{int}, HA^{low}, or with HA_{518–526} peptide-pulsed splenocytes. Their ability to lyse P815 (A–D), HA^{high} (E–H), HA^{int} (I–L), or HA^{low} targets (M–P) that were unpulsed (○) or pulsed with HA_{518–526} peptide ( ), was measured using a standard ^51Cr release assay. Data are representative of two experiments.
the Renca cell lines do not express either of these molecules (Fig. 2), other mechanisms by which HA-expressing tumor cells could directly activate naive clone 4 CD8+ T cells were investigated.

It has been suggested that ICAM-1 is able to provide T cells with a costimulatory signal in systems in which immobilized ICAM-1 protein or anti-LFA-1 Ab was used in conjunction with anti-TCR or anti-CD3 Abs (20–22). All of the Renca cell lines were shown to express ICAM-1 (Fig. 2), thus we wished to determine whether ICAM-1 interaction with LFA-1 was involved in the activation of clone 4 CD8+ T cells in vitro. The addition of an anti-ICAM-1 blocking Ab to the coculture system prevented clone 4 T cell activation by the HA-expressing tumor cell lines. This was demonstrated by the lack of increase in both forward scatter and CD69 expression (data not shown), and by the inhibition of IFN-γ production by clone 4 T cells (Fig. 7A). IFN-γ production was inhibited significantly in cultures in which the ICAM-1 blocking Ab was present. This was shown to be an Ab-specific effect as no significant reduction in IFN-γ production was seen in the presence of an isotype control Ab. The anti-ICAM-1 and isotype control Abs did not induce IFN-γ production by clone 4 T cells or by any of the tumor cell lines cultured alone (data not shown).

All of the Renca cell lines express ICAM-1, but not LFA-1 (Fig. 2). In contrast clone 4 T cells express both ICAM-1 and LFA-1 (data not shown). To determine whether the anti-ICAM-1 Ab was acting directly on the tumor cell lines and not on clone 4 T cells, thus discounting the possibility that homotypic adherence between T cells may be providing costimulation, a series of blocking experiments was conducted using an Ab against the CD11a subunit of LFA-1. Coculture of clone 4 T cells with HA-expressing tumor cell lines in the presence of the anti-CD11a Ab prevented clone 4 T cell activation, as demonstrated by the absence of an increase in both forward scatter and CD69 expression (data not shown). In addition, IFN-γ production by clone 4 T cells in response to all of the RencaHA tumor cells was significantly reduced (Fig. 7B). The reduction in IFN-γ production was Ab-specific, as in the presence of a control isotype Ab IFN-γ production was not significantly reduced. Furthermore, the presence of the anti-CD11a blocking Ab did not induce IFN-γ production by clone 4 T cells cultured alone or by tumor cells cultured alone (data not shown).

**Tumor-activated CTL inhibit tumor growth in vivo**

To determine whether the clone 4 CTL generated from in vitro coculture with HA-expressing tumor cell lines were able to affect the growth of tumor cells in vivo, BALB/c mice were injected s.c. with either the RencaNT or the Renca HAhigh tumor cell line (Fig. 8). Four days later mice each received 5 × 10⁶ clone 4 CTL generated in vitro coculture for 72 h with either the HAhigh tumor cell line or with HA518-526 peptide-pulsed splenocytes as a control. Other control groups of tumor-bearing mice were given either naive clone 4 T cells or no clone 4 T cells. Tumor growth in all recipient mice was monitored for 2 wk. In mice given RencaNT cells, the tumors grew at the same rate regardless of whether they had also received naive clone 4 T cells, or not, or clone 4 cells activated by coculture with either HAhigh tumor or HA518-526 peptide-pulsed splenocytes (Fig. 8). In mice that were injected with only the HAhigh cell line, tumor growth occurred at a similar rate as in mice given RencaNT cells, indicating that HA expression by the tumor cell did not affect its growth in vivo. The presence of naive clone 4 T cells had minimal effect upon the growth of the RencaHA tumor, whereas in mice that had received clone 4 CTL generated from coculture with HA518-526 peptide-pulsed splenocytes, the growth of the HAhigh tumor was impaired. Significantly, in mice that had been given clone 4 CTL generated from coculture with the HAhigh cell line, growth of the HAhigh tumor was completely inhibited.

**Tumor-activated clone 4 T cells persist in the periphery**

Current evidence indicates that T cell priming in the absence of CD28 signaling leads to a transient response with no memory formation (23). We investigated whether clone 4 T cells cocultured with tumor cells were able to survive for a prolonged period of time in vivo following ICAM-1/LFA-1-dependent stimulation in vitro. For this purpose clone 4 T cells (Thy1.1) were cocultured with either RencaHAhigh cells or HA518-526 peptide-pulsed splenocytes, then transferred into BALB/c mice (Thy1.2). All of the transferred clone 4 cells were activated as determined by CD69 staining (data not shown). Three weeks later mice were challenged with HA Ag in the form of influenza virus A/PR8/34. Six days after influenza challenge, peripheral lymph nodes were analyzed for the presence of Thy1.1 cells. Clone 4 Thy1.1 T cells activated via HA518-526 peptide-pulsed splenocytes (Fig. 9A) could be detected in challenged mice and constituted 0.059% of the peripheral lymph node population. Significantly, clone 4 Thy1.1 T cells activated in vitro on HAhigh tumor cells (Fig. 9B) via the ICAM-1/LFA-1-dependent mechanism also survived 27 days in vivo and similarly constituted 0.06% of the peripheral lymph node population following influenza virus challenge.

**Discussion**

The requirement for CD28-mediated costimulation in CD8+ T cell priming is not clear. It has been demonstrated that ligation of CD28 is required for CD8+ T cell activation in the generation of primary allogeneic responses in vitro (2). Alternatively priming of CD8+ T cells has been shown to occur in the absence of CD28 expression in several in vivo systems, including antiviral responses...
CD8+ T cells capable of IFN-γ production, lysis of tumor cell targets in vitro, and inhibition of tumor growth in vivo. Furthermore, tumor cell–primed T cells were able to persist in vivo in the absence of Ag and expand upon restimulation.

The inability of tumor cells in some systems to directly prime CD8+ T cell responses has been suggested to be due to an inability to provide costimulation. In systems in which direct priming by the tumor did not occur, priming could be induced by transfection of the tumor cells with B7 resulting in tumor rejection (27, 28). However, more recently it has been demonstrated that tumors expressing CD80 could not activate T cells in situ, but required the tumor cells to be present in the lymphoid compartment (16). This finding indicated that further stimuli are required for direct priming, which are unique to the lymphoid compartment.

In contrast, our system demonstrated that tumor cells alone can directly activate CD8+ T cells in vitro. Although this result suggests that direct priming of naïve CD8+ T cells could occur outside of the secondary lymphoid tissues, it is unlikely that this would happen as naïve T cells do not normally circulate through peripheral tissues. Therefore, although direct priming may be important in stimulating antitumor immunity this would only occur if the tumor entered the secondary lymphoid tissues. Ligation of tumor cell–primed T cells to LFA-1 was a requirement for direct CD8+ T cell activation. The interaction of ICAM-1 with LFA-1 may function in several ways to provide or facilitate costimulation.

First, it may simply increase adhesion between the tumor cell and T cell, thereby prolonging the duration of signal 1 through the TCR. Second, this interaction may provide an active signaling event to augment signal 1. Third, it may act to hold the cells in close proximity permitting a third interaction between the tumor cell and the T cell that otherwise would not occur.

In contrast to our findings with CD8+ T cells, costimulatory activity provided through LFA-1 on CD4+ T cells favored limited proliferation, apoptosis and anergy (20, 29, 30). This may indicate that CD4+ T cells have different costimulatory requirements to CD8+ T cells. We demonstrate in this study that the interaction of LFA-1 with ICAM-1 on the tumor is necessary for activating naïve CD8+ T cells to receive signal 2 and therefore may circumvent the requirement for CD80- and CD86-induced signaling through CD28. This is consistent with other reports in which Ag-independent stimulation of CD8+ T cells by anti-TCR Ab and ICAM-1 immobilized on beads was shown to induce cell proliferation and involved an active signaling event through LFA-1 that was distinct from TCR-mediated signals (21, 22). In addition, ICAM-1 was shown to be important for activation of CD28-deficient CD8+ T cells by anti-CD3 Abs (31). ICAM-1 expression is not unique to the Renca tumor cell line, but is expressed either constitutively or in response to inflammatory conditions on many cell types (32–34). Therefore, it is possible that ICAM-1-driven costimulation could be a major mediator of CD80/CD86-independent CD8+ T cell activation.

Significantly, direct activation of naïve clone 4 T cells by RencaHA cells did not result in their functional unresponsiveness but the generation of effector CTL, even when very low levels of endogenous tumor Ag was expressed. In contrast, the ability of tumor cell–activated clone 4 CTL to kill tumor cells in vitro was dependent on the level of HA Ag expressed by the target cell, with the efficiency of killing decreasing with lower levels of Ag expression. These data indicate that although the levels of MHC-Ag complexes on the tumor cell surface are sufficient for CD8+ T cell priming, they are insufficient to allow the tumor cell to act as a target of effector function. This corresponds with in vitro cytotoxicity assays of effector function in many systems, in which efficiency of target lysis is peptide dose-dependent (35). The inability of tumor-activated clone 4 CTL to kill Renca HA-low cells cannot be attributed to the CD28-independent mechanism by which they were primed, as a similar result was observed for CTL activated on HA (518–526) pulsed splenocytes, where presumably CD28-driven priming was occurring. Our results therefore suggest that tumor escape from immunoregulation may not be due to failure to prime CTL when Ag levels are low, but to a lack of susceptibility to killing by CTL at the effector stage.

Most importantly, clone 4 T cells directly activated by RencaHA tumor cells were able to inhibit the growth of the HA (high-expressing) cell line in vivo, when adoptively transferred early during tumor growth. In contrast, in mice that did not receive any clone 4 T cells, endogenous HA–specific T cells did not provide any protection against tumor growth. This was consistent with other findings in which only weak endogenous responses were detected against neotumor Ags and did not impact on tumor growth (36, 37). In addition, naïve clone 4 T cells adoptively transferred on day 4 of tumor growth were unable to provide protection against tumor growth in vivo. The inability of naïve clone 4 T cells to control tumor growth is not unique to this system (38, 39). The lack of protection may be due to a number of factors, including an absence of tumor Ag in the tumor draining lymph node at day 4 of tumor growth, resulting in ignorance and tumor expansion. Thus any subsequent CD8+ T cell activation may occur too late to control tumor growth. The transfer of in vitro–activated antitumor T cells may therefore be advantageous compared with in vivo activation. Firstly, transfer of T cells directly activated by tumor in vitro may avoid any problems associated with in vivo priming of naïve cells due to suboptimal Ag presentation. Secondly, T cells with specificities to epitopes not generated by cross–priming in vivo may be activated. Thirdly, direct-activation of naïve tumor-specific T cells in vitro appears to remove the problems associated with naïve T cell transfer, in that even though effector cells are generated, they do not always mediate tumor rejection.

Our findings confirm that naïve CD8+ T cells can be primed to become effector CTL in the absence of CD28 signaling. Under circumstances of normal T cell/APC interaction, ICAM-1–LFA-1 can substitute for CD28 allowing stimulation. Critically, these observations indicate that CD8+ T cell responses can be initiated independently of professional APC, and may instead result from...
presentation by nonprofessional APC such as virally infected cells or tumor cells. The fact that naïve T cells do not routinely migrate through peripheral tissues means that such presentation will only occur when tumor cells access lymphoid tissues (such as during metastasis), or when a virus infection involves replication in hematopoietic cells (for example, during infection with HIV or CMV). It is conceivable that direct priming by nonprofessional APC may not lead to the establishment of a prolonged reaction involving memory. Indeed, the stimulation of CD28-deficient CD8+ T cells using allogeneic stimulators was associated with only a transient response; an observation that was linked to a failure of the T cells to up-regulate the antiapoptotic protein Bcl-xL (23). Although these studies do not directly investigate the generation of memory, in our CD28-independent system we have demonstrated that CD8+ T cells activated by tumor cells in an ICAM-1/LFA-1-dependent manner are able to persist in the absence of Ag in vivo for nearly 4 wk.

Disclosures

The authors have no financial conflict of interest.

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