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Rejection of Allogeneic and Syngeneic But Not MHC Class I-Deficient Tumor Grafs by MHC Class I-Deficient Mice

Sofia Freland,* Benedict J. Chambers,* Malena Andersson,* Luc Van Kaer,† and Hans-Gustaf Ljunggren2*

The ability of TAP1−/−, β2m−/−, and TAP1/β2m−/− mice to mount rejection responses against allogeneic, syngeneic, and MHC class I-deficient tumor grafts was examined. The results demonstrate a potent ability of TAP1−/− and β2m−/− as well as TAP1/β2m−/− mice to reject allogeneic tumors. In contrast to published data, rejection of syngeneic MHC class I-expressing tumors was also observed. This response was specific for the MHC class I-deficient mice, since wild-type mice did not reject syngeneic MHC class I-positive tumors under identical experimental conditions. The rejection response of syngeneic tumors required preimmunization of the mice and was MHC class I specific at the level of priming as well as at the level of the tumor target. Finally, MHC class I-deficient tumor grafts were accepted in MHC class I-deficient mice while similar grafts were rejected in wild-type mice. In summary, while MHC class I-deficient mice have retained a capacity to reject allogeneic tumors, they have gained an ability to reject syngeneic MHC class I-positive tumors and lost the ability to reject MHC class I-negative tumors. The present results are discussed in relation to the role of MHC class I molecules in selecting functional CD8+ T and NK cell repertoires, and the development of cell-mediated immunity. The Journal of Immunology, 1998, 160: 572–579.

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3 Abbreviations used in this paper: β2m, β2-microglobulin; B6, C57BL/6 mice.

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tumors. In contrast to published data, we also observed rejection responses against syngeneic MHC class I-positive tumors in the mutant mice. Finally, in contrast to wild-type mice, MHC class I-deficient tumor grafts were accepted in MHC class I-deficient mice. The results are discussed in relation to the role of MHC class I molecules in selecting functional CD8+ T and NK cell receptors, and the consequences of this process for the development of a cell-mediated immune response in vivo.

Material and Methods

Mice

All mice were bred and maintained at the Microbiology and Tumor Biology Center (MTC), Karolinska Institute, Stockholm, Sweden. The generation of β2m−/− and TAP1−/− as well as MHC class II−/− mice has been described (4, 11, 24). β2m−/− mice were from the Jackson Laboratories (Bar Harbor, ME), β2m−/− of the H-2k and H-2d backgrounds were a kind gift from Dr. D. Roopenian. MHC class II−/− mice were a kind gift from C. Benoist and D. Mathis. TAP1/β2m−/− mice were obtained by intercrossing TAP1−/− and β2m−/− mice as described (15). All mice, when not otherwise noted, were on an H-2b background. The β2m−/− and TAP1−/− mice were back-crossed to C57BL/6 (B6) at least six times before use.

Tumors

P815 is a mastocytoma of DBA/2 (H-2b) origin. EL-4 is a T cell lymphoma of C57BL/6 (H-2b) origin. C4.4-25 is a β2m-deficient mutant of EL-4 (23). All tumors were maintained as tissue culture cell lines or passaged as ascites lines in 400-rad irradiated H-2 syngeneic mice.

Immunizations

For immunizations, mice were given 5 × 105 10,000-rad irradiated tumor cells or 25 × 103 3,000-rad irradiated spleen cells i.p. 1 wk prior to inoculation with live tumor cells.

Tumor rejection studies

Mice used for in vivo experiments were 6 to 10 wk old at the start of the experiments, usually littermates, or otherwise age matched within 2 weeks. Graded doses of tumor cells were inoculated s.c., in the left flank when not otherwise noted, in a 0.2-ml volume of PBS. Tumors always appeared at the site of inoculation. Tumor growth was followed at least once weekly by palpations and measurements of the tumor size. Mice were killed when the tumors reached a size of >15 mm in diameter, and no signs of rejection were observed. Mice without any signs of tumor growth were kept under observation for at least 6 wk after inoculation. Small groups of mice, never exceeding five mice per group, were tested in several independent experiments throughout the study to minimize the risk of random fluctuations in the quantity or quality of cells inoculated. Results from several independent tests were pooled if not otherwise noted.

Results

Efficient rejection of allogeneic tumors in TAP1−/−, β2m−/−, and TAP1/β2m−/− mice

B6 wild-type as well as TAP1−/−, β2m−/−, and TAP1/β2m−/− mice were grafted s.c. with titrated doses of P815 (H-2b) mastocytoma cells. B6 control mice did not show any signs of tumor growth when doses of 105 cells or less were grafted s.c. When 106 or 107 cells were grafted, tumors appeared at the site of inoculation in a fraction of mice inoculated but were rejected from all mice within 3 wk after inoculation (Fig. 1) (data not shown). A similar pattern was observed in TAP1−/− mice, although a relatively higher number of mice developed tumors after grafts of 106 or 107 cells. Nonetheless, complete tumor rejection responses were observed within 4 wk after tumor challenge in all mice studied (Fig. 1). In β2m−/− mice, tumors appeared at the site of inoculation after grafts of 104 tumor cells. Tumor rejection responses were similar or only slightly delayed as compared with TAP1−/− mice, and within 5 wk most mice had rejected grafted tumors. TAP1/β2m−/− showed a pattern similar to that of β2m−/− mice, with the exception of the lowest dose inoculated (105 tumor cells), when a majority of the mice developed tumors within 2 wk after tumor challenge. Yet, despite initial formation of large s.c. tumors (sizes up to 13 mm in diameter) nearly all TAP1/β2m−/− mice rejected the tumors within 5 wk after challenge (Fig. 1). Measurable differences in tumor sizes between the respective groups of mice were most clearly detectable within 2 wk after inoculation of 105 and 106 cells, when the relatively largest tumor loads were observed in TAP1/β2m−/− mice followed by β2m−/−, TAP1−/−, and B6 mice (Fig. 2).

MHC class I-expressing syngeneic tumors are accepted by untreated TAP1−/−, β2m−/−, and TAP1/β2m−/− mice

Previous studies with β2m−/− mice have demonstrated the generation of strong CD8+ cytotoxic responses in vitro against syngeneic MHC class I-positive cells, including tumor cells (9). However, despite these strong responses, β2m−/− mice do not reject syngeneic MHC class I-positive tumors in vivo (8, 10) (reviewed in Ref. 2). To address these apparently conflicting results, we first determined the growth of titrated (104–105) syngeneic MHC class I-expressing EL-4 cells in B6, TAP1−/−, β2m−/−, and TAP1/β2m−/− mice. These studies revealed a strikingly similar pattern of tumor growth in all MHC class I-deficient strains as well as in B6 wild-type mice (Fig. 3) (data not shown), confirming and extending published results in β2m−/− mice (8, 10). To exclude that these results were not a specific property of the B6-derived lymphoma EL-4, we performed similar titrations of MHC class I-expressing RMA lymphoma cells and MC57X fibrosarcoma cells (both B6 derived). These studies yielded results similar to those obtained with EL-4 cells (data not shown). To exclude that the present results were due to a general inability of the EL-4 cells to be rejected in β2m−/− mice, these cells were grafted to β2m−/− mice of the H-2d and H-2b haplotypes. In both of these strains, EL-4 cells were readily rejected after initial growth (Fig. 4; H-2d β2m−/− mice not shown), indicating that rejection responses could be mounted to the tumor even in mice of a β2m−/− background.

Simultaneous inoculation of allogeneic and syngeneic tumors fails to induce rejection of syngeneic tumors in MHC class I-deficient mice

In vitro studies have demonstrated that alloreactive CD8+ T cells from MHC class I-deficient mice generated in MLRs cross-react with syngeneic cells expressing normal levels of MHC class I molecules (9). This observation led us to address whether it was possible to induce rejection of syngeneic MHC class I expressing EL-4 cells by MHC class I-deficient (β2m−/−) mice upon simultaneous inoculation with allogeneic P815 cells. However, neither simultaneous inoculation of both tumors in different flanks in the same mouse nor intermixing the tumors prior to s.c. inoculation led to any significant rejection of the EL-4 tumor cells (Fig. 5). Mice in the latter group, inoculated with 1:1 mixtures of syngeneic EL-4 and allogeneic P815 tumors, readily developed s.c. tumors. When solid s.c. tumors were removed from these mice (at a tumor size of approximately 10 mm in diameter), all tumor cells were found to be H-2b positive and H-2d negative as revealed by FACS analysis, indicating selective growth of EL-4 tumor cells (data not shown).

Rejection of syngeneic tumors in MHC class I-deficient mice immunized with allogeneic tumor cells

In contrast to the observations above, we found that MHC class I-deficient (β2m−/−) mice that had previously rejected allogeneic P815 tumor grafts showed weak, albeit clearly detectable, rejection responses against EL-4 tumor grafts (Fig. 6) (data not shown). This result was specific for the MHC class I-deficient mice since EL-4
FIGURE 1. Allogeneic P815 tumor cells are rejected in TAP1<sup>−/−</sup>, β2m<sup>−/−</sup>, and TAP1/β2m<sup>−/−</sup> mice. Mice were inoculated s.c. with 10<sup>5</sup>, 10<sup>6</sup>, or 10<sup>7</sup> tumor cells, and the number of mice (percentage) with tumor growth was determined. In most experiments, mice developed tumors that subsequently regressed and disappeared. All graphs consist of pooled results from three to six independent experiments with three to six mice in each experiment.

FIGURE 2. Different growth of allogeneic P815 tumor cells in B6, TAP1<sup>−/−</sup>, β2m<sup>−/−</sup>, and TAP1/β2m<sup>−/−</sup> mice. Mean tumor load 2 wk after s.c. inoculation of 10<sup>5</sup> (A) and 10<sup>6</sup> (B) tumor cells. Data from Figure 1. Error bars indicate SE.
cells rapidly formed solid tumors with no signs of rejection in B6 wild-type mice that previously had rejected P815 tumor grafts. Similar results were obtained with β2m−/− mice that were preimmunized with irradiated P815 cells 1 wk prior to challenge with live EL-4 tumor cells (Fig. 6). These results mimicked previous in vitro results, in which allospecific CD8+ T cells from β2m−/− mice showed cross-reactivity on target cells expressing syngeneic class I molecules expressed at a normal ligand density (9) (reviewed in Ref. 2).

Rejection of syngeneic tumors in MHC class I-deficient mice preimmunized with syngeneic cells

The results above prompted us to preimmunize β2m−/− mice with irradiated syngeneic EL-4 tumor cells or B6-derived splenocytes and subsequently graft these mice with live EL-4 cells. While only relatively weak tumor rejection responses were observed in β2m−/− mice preimmunized with EL-4 cells, strong rejection responses were observed when β2m−/− mice were preimmunized with B6-derived spleen cells, and subsequently challenged with EL-4 cells (Fig. 7). Control experiments confirmed that the EL-4 cells were not rejected when grafted to B6 mice immunized with B6-derived spleen cells. Thus, the present observations demonstrate that MHC class I-deficient mice can reject tumor grafts expressing syngeneic MHC class I molecules, provided that they have been preimmunized. The relative differences observed in EL-4 rejection responses after immunization of β2m−/− mice with irradiated EL-4 cells and B6-derived splenocytes, respectively, could be due to either the cell dose used for immunization, the

FIGURE 3. Syngeneic EL-4 tumor cells are accepted in untreated TAP1−/−, β2m−/−, and TAP1/β2m−/− mice. Mice were inoculated s.c. with 10^2, 10^3, or 10^4 tumor cells, and the number of mice (percentage) with tumor growth was determined. In most experiments, mice developed tumors without visible signs of rejection responses. All graphs consist of pooled results from three to six independent experiments with three to six mice in each experiment.

FIGURE 4. EL-4 tumor cells are rejected in β2m−/− mice of the H-2k haplotype. Mice were inoculated s.c. with 10^5 tumor cells, and the number of mice (percentage) with tumor growth was determined. All mice developed tumors that subsequently regressed. The graph consists of pooled results from two independent experiments with five mice in each experiment.
more potent ability of spleen cells than tumor cells to prime T cells, or possibly other reasons. This matter was not investigated further.

Rejection of syngeneic tumors in MHC class I deficient mice is class I specific

The rejection responses observed against EL-4 cells in preimmunized MHC class I deficient mice were dependent on the expression of MHC class I molecules at the level of the target cell. Preimmunization of β2m−/− mice with either MHC class II−/− or control B6 wild-type splenocytes was sufficient to induce rejection of grafted EL-4 tumor cells, while preimmunization of β2m−/− mice with β2m−/− splenocytes did not induce rejection of grafted EL-4 tumor cells (Fig. 8). These responses were also dependent on the expression of MHC class I molecules on the cells used for immunization.
MHC class I-deficient mouse strains, including TAP1/2m mice, reveal a potent ability of MHC class I-deficient tumor grafts (summarized in Table I). The results and TAP1/2m mice demonstrated that MHC class I-deficient mice fail to efficiently kill MHC class I-deficient bone marrow grafts in vivo (17–21) (reviewed in Ref. 1). Earlier reports have discussed the origin of the CD8+ T cells detected in B2m−/− mice (reviewed in Ref. 2). It was speculated that the CD8+ T cells had the capacity to rapidly expand and react upon antigenic stimulation leading to rejection responses against allogeneic tumor cells. The present results extend these observations to TAP1−/− as well as TAP1/B2m−/− mice, and demonstrate that 1) numbers of CD8+ T cells in the different class I-deficient mice correlate with the ability to reject allogeneic tumor grafts, and 2) even very low numbers of CD8+ T cells, such as in the TAP1/B2m−/− mice (15), still are capable of rejecting large allogeneic tumor cell grafts.

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The present study has addressed the ability of TAP1−/−, B2m−/−, and TAP1/B2m−/− mice to reject allogeneic, syngeneic, and MHC class I-deficient tumor grafts (summarized in Table I). The results reveal a potent ability of MHC class I-deficient mouse strains, including TAP1/B2m−/− mice, to reject large grafts of allogeneic tumors. The ability to reject allogeneic tumor grafts correlated with numbers of CD8+ T cells in the mice. In contrast to the conclusions of previous reports, rejection responses against syngeneic MHC class I-expressing tumors were observed in MHC class I-deficient mice while similar responses were not detected in wild-type mice. Finally, MHC class I-deficient tumor grafts were accepted in MHC class I-deficient mice while similar grafts were rejected in wild-type mice.

Initial characterization of the B2m−/− mice, the first MHC class I-deficient mice generated, indicated a total lack of CD8+ T cells in the thymus and lymphoid organs and no detectable CD8+ T cell-mediated cytotoxicity (3, 4). As a consequence of these early reports, the B2m−/− mice were rapidly established as a model for assessment of various immune responses in the absence of CD8+ T cells (reviewed in Refs. 1 and 2). However, this notion was soon challenged. In the first published report with experimental tumor grafts, peritoneal exudate cells from B2m−/− mice that had been inoculated i.p. with allogeneic tumor cells contained a significant proportion of mature CD8+ T cells with MHC class I-specific cytotoxic activity (7). This report was followed by two other studies demonstrating that B2m−/− mice were capable of rejecting allogeneic tumors, while in vivo depletion of CD4+ or CD8+ T cells (or both) resulted in susceptibility to tumor growth (8, 10). In vitro experiments confirmed the presence of cytotoxic MHC class I-specific CD8+ T cells in these mice. Taken together, these studies indicated that the B2m−/− mice had low but clearly detectable numbers of CD8+ T cells, and that this pool of CD8+ T cells had the capacity to rapidly expand and react upon antigenic stimulation leading to rejection responses against allogeneic tumor cells. The present results extend these observations to TAP1−/− as well as TAP1/B2m−/− mice, and demonstrate that 1) numbers of CD8+ T cells in the different class I-deficient mice correlate with the ability to reject allogeneic tumor grafts, and 2) even very low numbers of CD8+ T cells, such as in the TAP1/B2m−/− mice (15), still are capable of rejecting large allogeneic tumor cell grafts.

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that these cells could develop either through positive selection on low levels of MHC class I molecules in the thymus or, alternatively, represent a small population of CD8\(^{+}\) T cells that differentiated independently of class I recognition. In an initial analysis of this phenomenon, it was observed that the small pool of CD8\(^{+}\) T cells selected in β2m\(^{-/-}\) mice not only reacted with the allogeneic cells but also cross-reacted and killed cells expressing self MHC class I expressed at normal levels (9). Similar findings were also observed in studies of TAP1\(^{-/-}\) and TAP1/β2m\(^{-/-}\) mice (12–15). This bias toward reactivity against syngeneic MHC class I expressed at a normal ligand density strongly indicated that at least a part of the residual CD8\(^{+}\) T cell population had been selected on the low levels of MHC class I molecules expressed in these mice. The studies in β2m\(^{-/-}\) mice also demonstrated that CD8\(^{+}\) T cells could be induced readily in a primary MLR after stimulation with H-2\(^{b}\) class I-positive cells, whereas allogeneic cells generally failed to elicit a response under similar conditions (9). In the latter case, in vivo priming was necessary for the generation of efficient responses. In retrospect, this observation may explain why Zijlstra et al. as well as Koller et al. in their initial studies failed to detect CD8\(^{+}\) T cell-mediated reactivity in the β2m\(^{-/-}\) mice (3, 4). Taken together, these results suggested that events occurring during T cell selection influence the reactivity of CD8\(^{+}\) T cells selected in MHC class I-deficient mice (9) (reviewed in Ref. 2). These findings have been taken as evidence for a novel specificity of the CD8\(^{+}\) T cells in MHC class I-deficient mice, i.e., an ability to specifically recognize and kill target cells expressing syngeneic MHC class I expressed at a normal ligand density (9, 12). Similar conclusions have more recently been made from studies in mice expressing MHC class II molecules loaded with a single or a limited set of peptides (27–30).

The findings that MHC class I-deficient mice can generate CD8\(^{+}\) T cell responses against cells expressing syngeneic MHC class I also provides an explanation for earlier experiments with β2m\(^{-/-}\) mice using skin grafts (31, 32). These mice were shown to reject syngeneic MHC class I-positive skin grafts. The discovery of a residual CD8\(^{+}\) T cell pool in the β2m\(^{-/-}\) mice, and the strong bias of these CD8\(^{+}\) T cells toward reactivity with syngeneic MHC class I molecules expressed at a high ligand density, suggests that CD8\(^{+}\) T cells could contribute to the rejection response against syngeneic MHC class I-positive skin grafts in the β2m\(^{-/-}\) mice. This interpretation was also used to explain the rejection of MHC class I-positive skin grafts in TAPI/β2m\(^{-/-}\) mice (15). However, the idea of a self-biased CD8\(^{+}\) T cell repertoire in β2m\(^{-/-}\) mice was not fully compatible with the notion of the inability of β2m\(^{-/-}\) mice to reject H-2\(^{b}\)-expressing tumor grafts (8, 10). In a more detailed analysis of this phenomenon, Jhaver et al. suggested that these findings could be explained by defects of the CD8\(^{+}\) T cells from β2m\(^{-/-}\) mice to proliferate and secrete cytokines (e.g., IFN-γ or IL-3/γ/δ-granulocyte-macrophage-CSF) upon stimulation with targets expressing normal levels of self MHC class I molecules (33). This led them to suggest that the CD8\(^{+}\) T cells from β2m\(^{-/-}\) mice are in a state of partial but not complete tolerance (i.e., split tolerance), leading to an inability to reject H-2\(^{b}\) tumors while generating strong cytotoxic activity against the same tumors in vivo. To explain the discrepancy in the ability to reject skin grafts but not tumor grafts, it was speculated that generation of cytotoxic responses might be sufficient to reject skin grafts despite no (or only moderate) proliferative responses, whereas eradication of rapidly growing tumors may require additional mechanisms such as the ability to rapidly proliferate and secrete cytokines (33). The results in the present study indeed confirm that naive β2m\(^{-/-}\) mice are unable to reject syngeneic MHC class I-positive grafts, yet they demonstrate that preimmunization (with either allogeneic or syngeneic cells) readily primes the mice to reject syngeneic MHC class I-expressing tumor grafts. Thus, the present observations suggest that the state of “split tolerance” toward MHC class I-positive tumor grafts in MHC class I-deficient mice may be relative rather than absolute.

Our observation that MHC class I-deficient mice accept MHC class I-deficient tumor grafts agrees well with the previous findings that these mice accept MHC class I-deficient bone marrow grafts (17, 20, 21). However, the present results do not exclude the possibility that MHC class I-deficient mice, such as the β2m\(^{-/-}\) strain, can kill β2m\(^{-/-}\) or other MHC class I-deficient cells under certain conditions. Indeed, Höglund and collaborators have recently demonstrated that purified NK cells from β2m\(^{-/-}\) mice can selectively discriminate between MHC class I-positive and class I-deficient tumor cells in vitro, and selectively kill the latter (P. Höglund, personal communication).

In conclusion, the present study illustrates how an MHC class I-deficient environment affects the ability of mice to respond to allogeneic, syngeneic, and MHC class I-deficient tumor grafts. The mice have retained an ability to reject allogeneic tumors; they have acquired an ability to reject syngeneic MHC class I-positive tumor grafts and, in contrast to wild-type mice, have lost the ability to reject MHC class I-deficient tumors.

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