Brief Definitive Report

Tumorigenicity Conferred to Lymphoma Mutant by Major Histocompatibility Complex-encoded Transporter Gene

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Summary

Presentation of antigenic peptides by major histocompatibility complex (MHC) class I molecules requires MHC-encoded molecules of the adenosine triphosphate binding cassette (ABC) family. Defects in these proteins represent a potential risk, since they are essential links in the machinery of T cell–mediated surveillance which continuously scrutinizes peptide samples of cellular proteins. Nevertheless, transfection of the mouse lymphoma mutant RMA-S with the rat ABC gene *mtp2*^{a} (homologue to mouse *HAM2* and human *RING11*), commonly termed *TAP-2* genes, led to a marked increase in tumor outgrowth potential in vivo. This occurred despite restored antigen presentation and sensitivity to cytotoxic T lymphocytes, and was found to be due to escape from natural killer (NK) cell–mediated rejection. It has previously been proposed that adequate expression of self-MHC class I is one important mechanism to avoid elimination by NK cells. Our data argue that a defect in the machinery responsible for processing and loading of peptides into MHC class I molecules is sufficient to render cells sensitive to elimination by NK cells. The latter thus appear to function as a surveillance of the peptide surveillance machinery.

MHC class I molecules present short antigenic peptides to CD8$^+$ cytotoxic T cells (1–3). The peptides presented are derived from cytosolic proteins, usually translated endogenously (2). There are at least two genes in the MHC that code for products involved in peptide supply to MHC class I molecules, termed *TAP-1* and *TAP-2*. These products are homologous to the transport proteins in the ATP binding cassette (ABC) family, and are thought to mediate transport from the cytoplasm to the endoplasmic reticulum, either of peptides or a cofactor essential for peptide loading into MHC class I molecules (4–13). The mouse lymphoma mutant RMA-S has a profound (14–16), but not total (17–19), peptide presentation defect in the MHC class I presentation pathway, which is due to a premature stop in the message of the mouse *TAP-2* gene *HAM2* (19a). The phenotype was restored to normal by transfection of *TAP-2* genes from different species, first the *mtp2*^{a} rat gene (9) and then the *HAM2* mouse gene (12) and the *RING11* human gene (A. Townsend, personal communication); the *mtp2*^{a} transfectant (RMA-S.mtp2^a) was able to process and present influenza virus epitopes to CD8$^+$ T cells (9). This transfectant system has now made it possible to investigate directly the role of TAP genes for in vivo phenomena such as tumorigenicity and transplant rejection. The aim of the present study was to investigate the effects on in vivo immunobiology of RMA-S after restoration of its antigen presentation defect by the rat *TAP-2* gene.

Materials and Methods

Animals. C57BL/6 mice were bred and maintained at the Department of Tumor Biology, Karolinska Institutet. Athymic nu/nu C57BL/6 mice were purchased from Bomholtgaarden, Denmark. All mice were 4–8 wk old at the start of the experiments.

Tumor Cell Lines. RMA (derived from RBL-5 after mutagenization, nonselected [20, 21]), RMA-S (derived from RBL-5 after mutagenization and selection with anti-H-2^b antiserum plus complement for loss of MHC class I expression [20, 21]), and RMA-S.mpt2^a (RMA-S transfected with *mtp2*^{a} cDNA; a neomycin-resistant expression vector driven by the β-actin promoter [9]) were grown in RPMI 1640 supplemented with penicillin and streptomycin plus 5% FCS and kept in 50-ml culture flasks at 37°C, 5% CO_2. The tissue-cultured medium of RMA-S.mtp2^a was supplemented with G418 1 mg/ml to maintain the plasmid.

In Vivo Rejection Assay. Untreated and NK1.1 antibody-treated (0.2 ml NK1.1 ascitic fluid intraperitoneally, days −1 and +5) syngeneic C57BL/6 mice were used. Tumor cells were inoculated subcutaneously either in separate flanks or in separate animals, and outgrowth was monitored by palpations twice weekly. Tumors appeared at the site of inoculation, grew progressively, and subsequently killed the mice. In vitro tumor cell lines, phenotypically indistinguishable from the injected tumor cell line, could regularly

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be established from these tumors (data not shown). Macroscopically, metastases were not observed. However, if single cell suspensions were prepared from different organs, e.g., spleen, in vitro tumor cell lines could occasionally be established, which were phenotypically indistinguishable from the injected tumor cell line (data not shown). After initial experiments, the mice were killed when the mean tumor diameter reached 15 mm and no signs of regression were seen. The tumors were examined macroscopically after killing the animals. Mice without tumor growth were observed for at least 8 wk after inoculation.

Short-Term In Vivo Rejection Assay with ^{125}I-UdR-labeled Cells. Untreated and NK1.1 antibody-treated (0.2 ml NK1.1 ascitic fluid intraperitoneally 24 h before the test) syngeneic C57BL/6 mice were used. 20 × 10^6 tumor cells (RMA, RMA-S, and RMA-S.mtp2^a, respectively) were incubated in 20 ml RPMI plus 5% FCS plus 10 μCi ^{125}I-UdR at 37°C for 5 h, washed three times, and diluted to a final concentration of 5 × 10^6 cells/ml. 200 μl (10^6 cells) of the cell preparations was injected intravenously. The mice were killed after 8 h and the remaining radioactivity was measured in the lungs, the liver, the spleen, and the kidneys, in a gamma counter (Pharmacia-LKB Biotechnology, Stockholm, Sweden). The arithmetic means and SD in each group (five mice/group) from one representative experiment are given.

In Vitro Cytotoxic Assay with Antibody plus Complement-depleted Effector Cell Populations. NK cell activity of syngeneic C57BL/6 mice was boosted by administrating 0.2 ml of a 10 mg/ml preparation of tilorone analogue R10.874 DA (Sigma Chemical Co., St. Louis, MO) per os to each animal on day −1. Tilorone augments NK cell activity by induction of IFN in the host (22). Single cell suspensions of spleens from the tilorone-boosted mice were prepared in tissue culture medium. Aliquots of 20 × 10^6 cells were incubated with 1.5 ml of mAb for 45 min, washed twice in PBS, incubated in 1.5 ml of rabbit complement for 60 min at 37°C, 5% CO_2, washed twice in PBS, and finally diluted in tissue culture medium. Reagents used were rabbit complement (Pel-Freez, Brown Deer, WI) diluted 1:8, anti-CD4 (RM-4-4; PharMingen, Scan Biotech, Falkenberg, Sweden) diluted 1:100, anti-CD8 (YTS 169.4; Sera-lab, Kemila, Stockholm, Sweden) diluted 1:500, and anti-asialo-GM1 (Wako Chemicals, Düsseldorf, Germany) diluted 1:100. Dilutions were made in tissue culture medium, and the preparations were used as effectors. RMA, RMA-S, and RMA-S.mtp2^a were used as targets. A standard 4-h ^{51}Cr-release assay was performed as previously described (23) with the effector/target ratios 100:1, 33:1, and 11:1.

Results and Discussion

RMA-S.mtp2^a had a somewhat lower growth rate in vitro as compared with RMA-S (data not shown). Despite this, the transfectant had acquired a malignant phenotype. In contrast with the mutant, the *mtp2^a* transfectant grew out in the majority (70%) of animals after subcutaneous inoculation of 10^3 cells (Fig. 1 *A*). The mutant failed to grow in more than 20% of the animals even after a 100-fold increase of the inoculum to 10^5 cells, a dose that resulted in 100% tumor outgrowth of transfectant cells (Fig. 1 *B*). The difference in tumorigenicity between mutant and transfectant cells persisted in T cell–deficient nude mice (data not shown), but disappeared in animals treated with anti-NK1.1 antibodies (Table 1). The tumorigenicity of the transfectant was due to its capacity to escape from rapid elimination by NK cells.

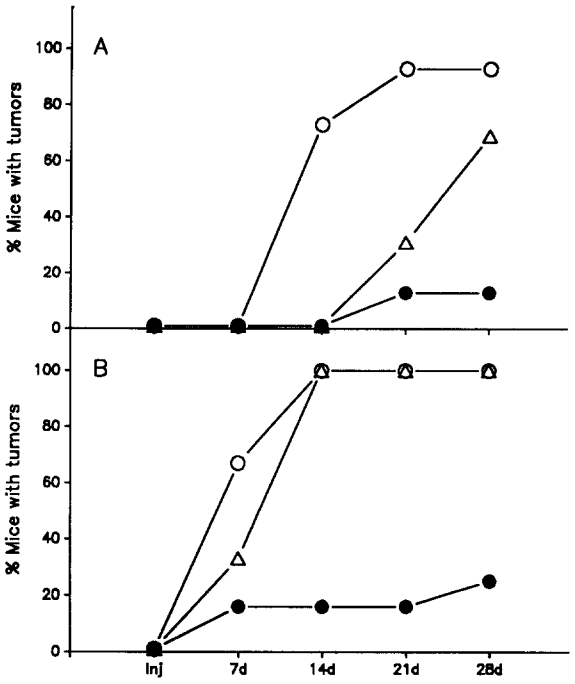

Figure 1. In vivo outgrowth of RMA (○), RMA-S (●), and RMA-S.mtp2^a (△) lymphoma cells in syngeneic C57BL/6 mice. (*A*) 10^3 tumor cells injected subcutaneously and outgrowth monitored by palpations twice weekly; (*B*) 10^5 tumor cells injected subcutaneously and outgrowth monitored by palpations twice weekly. Means of three separate experiments, with a total of 12–15 mice in each group are given.

Radiolabeled, intravenously inoculated transfectant cells survived well in the lungs (Fig. 2) and liver (data not shown) in normal as well as in NK1.1-treated animals, whereas the mutant cells without *mtp2^a* survived only in the latter. The *mtp2^a* transfection also rendered RMA-S resistant to NK cells in vitro (Fig. 3, *A* and *B*).

To our knowledge, this change in tumor cell rejectability is the first direct demonstration that a MHC-encoded transporter gene can have a decisive influence on disease progression in vivo. The result may seem surprising, since one might

Table 1. *In Vivo Outgrowth of RMA-S and RMA-S.mtp2^a Lymphoma Cells in Untreated and NK1.1 Pretreated Syngeneic C57BL/6 Mice*

	Host untreated		Host NK1.1 pretreated	
Cells injected	RMA-S	RMA-S. mtp2^a	RMA-S	RMA-S. mtp2^a
Separate flanks	0/8	7/8	8/8	8/8
Separate animals	3/13	15/15	14/15	15/15

In vivo outgrowth of 10^5 RMA-S and RMA-S.mtp2^a lymphoma cells in untreated and NK.1 antibody-treated syngeneic C57BL/6 mice after subcutaneous inoculations.

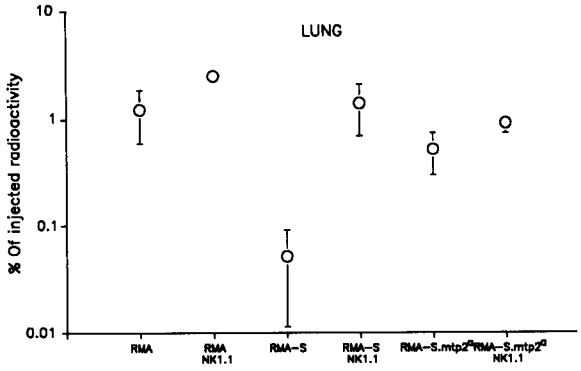

Figure 2. Short-term in vivo survival of ^{125}I-UdR-labeled RMA, RMA-S, and RMA-S.mtp2^a lymphoma cells in untreated and NK1.1 antibody-treated syngeneic C57BL/6 mice. 10^6 tumor cells were injected intravenously and the remaining radioactivity in the lungs was determined after 8 h. The arithmetic means and SD in each group from one representative experiment are given.

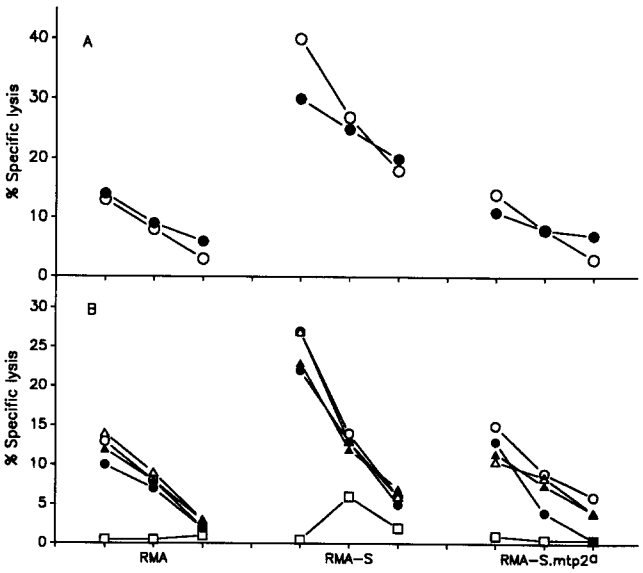

Figure 3. Sensitivity of RMA, RMA-S, and RMA-S.mtp2^a to syngeneic C57BL/6 and allogeneic BALB/c NK effector cells. (*A*) Syngeneic C57BL/6 (○) and allogeneic BALB/c (●) NK effector cells were tested at effector/target ratios 300, 100, and 33:1. (*B*) Syngeneic C57BL/6 spleen effector cells were pretreated either with C′ (●), anti-CD4+ C′ (△), anti-CD8+ C′ (▲), anti-asialo-GM1+ C′ (□), or left untreated (○), and tested at effector/target ratios 300, 100, and 33:1.

expect a restored antigen presentation to benefit the host rather than the tumor. However, the present system involves NK cells as important antitumor effector cells, and the outcome may be different when T cells dominate the scene. The low malignancy and the NK sensitivity of RMA-S has been reported previously (20, 21, 24, 25), but it was not known whether any of these properties was directly related to the defect in MHC-encoded transporter genes. From other experimental systems, it is clear that correction for a H chain gene or β_2-microglobulin (β_2m) gene defect can also be sufficient to restore a phenotype permitting escape from NK cells in vivo (26–28). In vitro studies of NK sensitivity have shown the same pattern (29–33). The component in the MHC class I molecule that is critical for protection against NK recognition remains to be identified, even if data from site-directed mutagenesis experiments strongly imply a role for peptides and/or the peptide binding groove of the H chain (31, 34). The present results are in line with this idea.

There is a mutual stabilizing effect of peptide and β_2m binding to the H chain. Conversely, dissociation from the H chain of either peptide or β_2m rapidly leads to irreversible loss of the tertiary structure and the antigen binding cleft (14, 35–39). Thus, the molecular machinery fixed by evolution for the MHC class I pathway provided a peptide-carrier system under considerable dissociative stress. This reduces the risk of binding novel peptides after the molecule has reached the cell surface, which is essential to avoid "innocent bystander killing" of normal cells binding external peptides. Another consequence is that any defect leading to impaired antigen presentation, be it in the H chain (26, 27), β_2m (28) or supply of peptides, will lead to reduced cell surface expression of H chain and a NK-sensitive phenotype. In light of our finding that a defect in one gene product in the processing machinery is sufficient to make a cell target for rapid elimination, it appears that NK cells fulfill one important proofreading/scavenger function necessary for full efficiency of T cell surveillance.

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