

SELECTIVE ACCEPTANCE OF MHC CLASS I-DEFICIENT  
TUMOR GRAFTS IN THE BRAIN

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For decades the brain was considered an immunologically privileged site (1). More recently, it has been realized that T cell-mediated allograft rejection and other immune responses can take place within the brain (2-5). The unusually low expression of MHC class I molecules in this organ (6, 7) is particularly interesting in relation to recent findings where a T cell-independent but NK cell-dependent defense, directed selectively against cells with reduced or deficient expression of MHC class I molecules, has been suggested (8-11). We have asked whether such a defense, possibly discriminating self from "no self," is allowed to work in the brain. The rejection potential against H-2-deficient tumor variants could not be detected in the brain, and our results suggest a selective difference between T cell- and NK cell-dependent immunity extracranially and intracranially.

Material and Methods

*Animals.* All inbred mouse strains used in this study were bred and maintained at the Dept. of Tumor Biology, Karolinska Institute.

*Tumor Cell Lines.* RBL-5 is a Rauscher virus-induced T cell lymphoma of C57BL/6 origin. The selection and subsequent phenotypic analysis of the RBL-5 H-2-deficient variants have been described in detail elsewhere (9). All tumor cell lines used were routinely passaged as ascites lines in H-2 identical or syngeneic F<sub>1</sub>-hybrid mice, preirradiated (400 rad) 24 h before tumor inoculation to avoid immunoselection. The following nomenclature of the tumor cell lines is used in this study: *RMA* for the mutagenized but not anti-H-2 selected RBL-5 lymphoma line. *RMA-S* and *RMB-S* for the two independently mutagenized and anti-H-2 selected sublines. These lines have previously (9-11) been called *RMA H-2 sel* and *RMB-S H-2 sel*.

*Tumor Growth Experiments.* Data from the two independently selected H-2-deficient lines were similar and were pooled in Table I. The different doses of ascitic tumor cells were inoculated in 4-8-wk-old animals, three to six mice per group, usually littermates or otherwise age matched within 2 wk in several independent experiments, and the subsequent data were pooled. Mice inoculated intracerebrally or intravenously were observed daily after inoculation and were kept until they died in the initial experiments. In subsequent studies, the mice were killed and autopsied when physiological or neurological signs indicated that they would have died within 24 h because of the tumor burden. Mice

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inoculated in the brain died from intracerebral lymphoma, intravenous inoculated mice developed generalized lymphoma with massive infiltration of liver and lungs. Mice inoculated subcutaneously or intraperitoneally were observed at least twice weekly. For intracerebral inoculation, tumor cells were inoculated in a 10- $\mu$ l volume into the right hemisphere with a microinjector (100- $\mu$ l type; Hamilton Co., Reno, NV) and a Yaoui needle. Surgical thymectomy was performed on 3–4-wk-old anesthetized animals. 3 wk later, the thymectomized mice (Thx) and control littermates (Thx-sham) received 735 rad whole body irradiation and were reconstituted by 5–15  $\times$  10<sup>6</sup> i.v. inoculation of syngeneic fetal liver cells. Immunized mice were prepared by three subcutaneous inoculations with 10<sup>7</sup> preirradiated (10,000 rad) RMA H-2<sup>+</sup> tumor cells with 1-wk intervals.

**FACS Analysis.** For FACS analysis, tumors growing in the brain were removed under sterile conditions and single cell suspensions were made. The tumor cells were subsequently put into tissue culture medium (RPMI + 5% FCS supplemented with antibiotics) for ~3–5 d and then analyzed on the FACS as described in reference 9. The mAbs were the same as described previously (9).

**Rapid Elimination Assay.** The rapid elimination assay with <sup>125</sup>I-IUdR-labeled cells was performed as described (9).

## Results and Discussion

**Intracerebral Growth of H-2<sup>+</sup> and H-2<sup>-</sup> Tumor Grafts.** The selective elimination of subcutaneous or intravenous inoculated RBL-5 H-2<sup>-</sup> lymphoma cells (9, 11) was markedly altered in the brain. When H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells were inoculated into the brain, no difference in either tumor take (Table I) or survival time (data not shown) was seen. In contrast, the H-2<sup>-</sup> cells required a 10<sup>3</sup>–10<sup>4</sup>-fold higher dose, compared with the H-2<sup>+</sup> cells, to induce >50% tumor take after subcutaneous, intravenous, or intraperitoneal inoculation in control mice (Table I). The changed pattern after intracranial inoculation was not just due to a different threshold dose for tumor inocula in general at this site. A wide range of tumor doses were tested and there was never any difference in intracerebral growth between the H-2<sup>+</sup> and the H-2<sup>-</sup> cell lines.

Pathological examination of the H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells, growing in the brain, showed no difference. Both tumor cell lines grew equally well at the site of inoculation with extension along the needle track to the subarachnoidal space (data not shown).

When the H-2<sup>+</sup> and the H-2<sup>-</sup> cell lines were mixed in vitro in a 1:1 ratio and inoculated intraperitoneally or subcutaneously in small doses in syngeneic mice, only H-2<sup>+</sup> tumor cells grew out and formed progressively growing ascites or solid tumors (12). When the same 1:1 mixture of H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells was inoculated in the brain, the output tumor cell population consisted of both H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells, on average in a 1:1 ratio. This difference in tumor cell outgrowth intracerebrally and intraperitoneally was seen even if the *same* animal was inoculated at both locations simultaneously (Fig. 1). This important control indicated that the intracerebral inoculation per se had no systemic suppressive effect on the host resistance against H-2<sup>-</sup> cells; the H-2<sup>-</sup> cells were always eliminated selectively from the mixture at the extracranial sites.

**No Rapid Elimination of H-2<sup>-</sup> Cells in the Brain.** The difference in extracranial tumor growth between the H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells was previously attributed to a rapid elimination mechanism taking place within 24 h after inoculation of radiolabeled cells (9). In light of the results presented above, it could then be

TABLE I  
Tumor Growth after Intracerebral, Subcutaneous, Intraperitoneal, and Intravenous  
Inoculation of RBL-5 H-2<sup>+</sup> and H-2<sup>-</sup> Tumor Cells

Mice	Inoculation dose	Number of mice with tumor growth/total number of mice inoculated							
		Intracerebral		Subcutaneous		Intraperitoneal		Intravenous	
		H-2 <sup>+</sup>	H-2 <sup>-</sup>	H-2 <sup>+</sup>	H-2 <sup>-</sup>	H-2 <sup>+</sup>	H-2 <sup>-</sup>	H-2 <sup>+</sup>	H-2 <sup>-</sup>
C57BL/6	10 <sup>1</sup>	0/5	0/5	—	—	—	—	—	—
	10 <sup>2</sup>	0/8	0/8	4/14	0/9	12/12	1/11	11/19	0/13
	10 <sup>3</sup>	10/12	12/12	11/14	1/27	11/12	0/14	9/11	0/13
	10 <sup>4</sup>	10/10	11/11	36/36	3/59	4/4	4/13	9/10	2/14
	10 <sup>5</sup>	7/7	14/14	27/27	8/36	10/10	5/16	9/10	7/10
	10 <sup>6</sup>	—	—	8/8	12/12	5/5	4/5	10/10	10/10
A/Sn	10 <sup>4</sup>	1/8	7/8						
A/Sn Thx	10 <sup>4</sup>	6/6	—						
A/Sn Thx-sham	10 <sup>4</sup>	0/4	3/4						
CBA	10 <sup>4</sup>	1/4	7/8						
C57BL/6 H-2 <sup>+</sup> immunized	10 <sup>4</sup>	0/4	4/4						

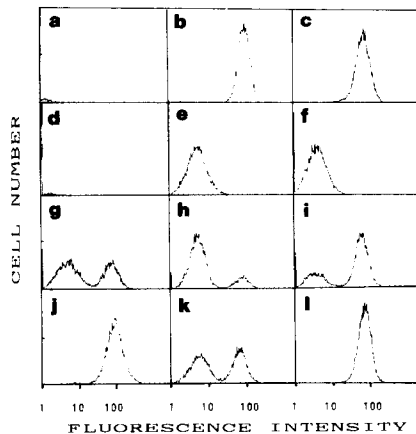


FIGURE 1. FACS analysis of RBL-5 H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells indirectly stained with mabs against H-2K<sup>b</sup> and H-2D<sup>b</sup>. (a-c) The H-2<sup>+</sup> RMA wild-type line; (a) nonspecific background staining with secondary antibody alone; (b) expression of cell surface H-2 antigens after serial passage in 400 rad-irradiated animals; (c) expression of cell surface H-2 antigens after progressive growth in the brain. (d-f) The RMA-S H-2<sup>-</sup> variant; (d) nonspecific background staining; (e) expression of cell surface H-2 antigens after serial passage in 400 rad-irradiated animals; (f) after progressive growth in the brain. (g-i) Three representative curves of cell suspensions from intracerebrally growing tumors where the H-2<sup>+</sup> and H-2<sup>-</sup> line were mixed in a 1:1 ratio before intracerebral inoculation. In most experiments the progressively growing intracerebral tumor consisted of both tumor cell phenotypes (H-2<sup>+</sup> and H-2<sup>-</sup>) in equal amounts as illustrated in g. In some mice one of the tumor cell populations

dominated as illustrated in h and i. The two cell surface phenotypes were equally often over represented (data not shown). (j) An ascites tumor from one representative mouse inoculated intraperitoneally with a 1:1 mixture of H-2<sup>+</sup> and H-2<sup>-</sup> cells. The intraperitoneal rapidly growing ascites cell line consists of only H-2<sup>+</sup> tumor cells. (k and l) Intracerebral and intraperitoneal inoculation of a 1:1 mixture of H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells in the same mouse; (k) intracerebrally, both the H-2<sup>+</sup> and the H-2<sup>-</sup> cells grew equally well (l) intraperitoneally, only H-2<sup>+</sup> cells grew. In all these experiments 10<sup>4</sup> cells were inoculated in normal syngeneic C57BL/6 mice. A logarithmic scale for fluorescence was used to visualize the H-2-deficient (H-2<sup>-</sup>) tumor cell line.

predicted that this rapid elimination should not take place in the brain. To test this, we inoculated radiolabeled H-2<sup>+</sup> and H-2<sup>-</sup> cells intracerebrally, subcutaneously in the hind leg, and intravenously in normal syngeneic mice. No difference in rapid elimination was seen between H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells in the brain 24 h after grafting of radiolabeled cells. In contrast, intravenously inoculated control mice showed an up to 100-fold difference in clearance between H-2<sup>+</sup> and H-2<sup>-</sup> tumor cells, with the most efficient elimination of H-2<sup>-</sup> cells in the lungs, followed by the liver and the spleen (reference 9, Fig. 2). There was also a more than fivefold difference in elimination between the H-2<sup>+</sup> and the H-2<sup>-</sup> tumor cells after subcutaneous inoculation (Fig. 2).

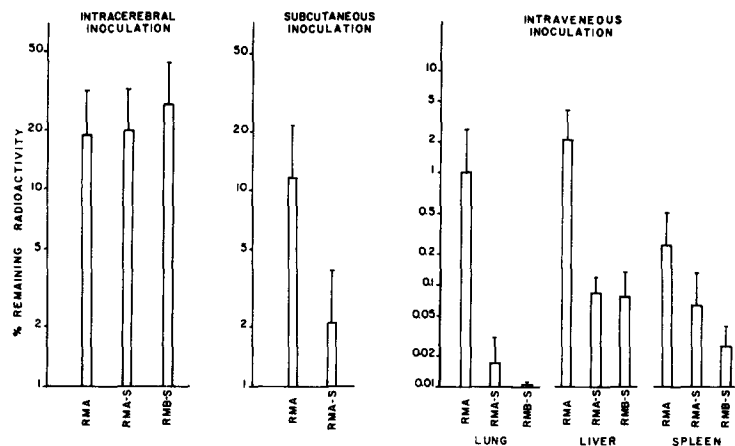


FIGURE 2. Survival of  $^{125}\text{I}$ -IUdR-labeled  $\text{H-2}^+$  (RMA) and two  $\text{H-2}^-$  (RMA-S and RMB-S) RBL-5 tumor cells in syngeneic C57BL/6 mice 24 h after intracerebral, subcutaneous, or intravenous inoculation. The amount of radioactivity remaining in the head of intracerebrally inoculated animals, in the hindleg of subcutaneously inoculated animals, and in the lung, liver and spleen of intravenously inoculated animals indicates the amount of surviving tumor cells. The results were pooled from four independent experiments with intracerebral inoculation, seven independent experiments with subcutaneous inoculation, and two independent experiments with intravenous inoculation, three to eight mice in each group, usually littermates or otherwise age matched within 2 wk. Figure shows arithmetic mean and SD on a log scale.

*Demonstration of Graft Rejection Against  $\text{H-2}^+$  Cells in the Brain.* The present data, demonstrating a selective acceptance of  $\text{H-2}^-$  cells in the brain, are not in conflict with previous evidence for immune reactions in the central nervous system (CNS) (2–5, 13). In the same experimental system as above we could confirm classical T cell-mediated rejections in the brain. When  $10^4$   $\text{H-2}^+$  cells were inoculated intracerebrally they grew out in all normal syngeneic mice, whereas most allogeneic or syngeneic preimmunized mice (immunized with the RBL-5  $\text{H-2}^+$  tumor) rejected them. If allogeneic mice were thymectomized, irradiated, and reconstituted before intracerebral inoculation of  $\text{H-2}^+$  cells, no rejection was seen. This indicated that the rejection of  $\text{H-2}^+$  cells in the brain was T cell dependent (Table I). The observation that the  $\text{H-2}^-$  cells grew even in allogeneic or preimmunized mice was consistent with the known importance of MHC class I molecules in allogeneic ( $\text{H-2}$ -specific) and syngeneic ( $\text{H-2}$ -restricted) immune responses (14).

*Conclusions.* The selective rejection of MHC class I-deficient cells extracranially was previously shown to be dependent on NK cells (9–11). Further in vitro analysis has suggested that NK cells distinguish between  $\text{H-2}^+$  and  $\text{H-2}^-$  cells in this system at a postbinding stage of the effector–target interaction (12). Our data suggest that mechanisms might have evolved to prevent rejection of MHC-deficient cells in the brain, either by suppression of effector cell activity or migration into the brain.

In tumor biology, the present findings may contribute knowledge to the particular patterns of metastasis observed in relation to the CNS. Several leukemias and solid tumors can spread and grow intracerebrally, while primary brain tumors seldom metastasize extracranially.

From a more general immunological point of view, it is important to identify the cells that can contribute to tissue damage during immune responses in the CNS (5, 15). The rapid NK responses during viral infections could cause an unnecessarily large irreversible damage of the nervous tissues and the brain in particular. This activity might thus be locally prevented in favor of slower and milder mechanisms for virus clearance (13).

In transplantation immunology, the present data are relevant to a peculiar type of rejection seen against hematopoietic grafts. In addition to the conventional T cell-mediated responses, allogeneic grafts as well as semisyngeneic (parental into F<sub>1</sub> grafts) are often rapidly rejected by a thymus-independent radioresistant mechanism (16, 17). It has been hypothesized that this surveillance system might be directed against cells with a partial or complete absence of H-2 *self* markers of the host (8, 10). If these types of rejections are mediated by the same NK-dependent system that rapidly rejects H-2<sup>-</sup> grafts extracranially, one would expect them to be turned off in the brain. There are indications that this may be the case (18).

The absence or suppression of the host defense against MHC class I deficient cells in the brain could also be relevant for the rapidly growing interest in the grafting of normal, neuronal, or other cellular components into the brain.

### Summary

H-2-deficient (H-2<sup>-</sup>) tumor variants were accepted equally well compared with H-2<sup>+</sup> wild-type cells in the brain of syngeneic mice, while the H-2<sup>-</sup> cells were selectively eliminated when inoculated extracranially. This indicates a specific absence or suppression of the defense against MHC class I-deficient cells in the brain, suggested to be mediated by NK cells. In contrast, T cell-mediated immune reactions could clearly be detected in the brain under the same experimental conditions. This was shown in control experiments where H-2<sup>+</sup> tumor cells were rejected from the brain of preimmunized or allogeneic mice. The present findings may be important for the understanding of neurotropic virus infections, immunology and immunotherapy of brain tumors, as well as for the growing interest in tissue grafting within the central nervous system.

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