Possible role of $Ab^b$ gene in mouse resistance to EL4 metastases

Olga S. Egorov, Yinong Liu, and Igor K. Egorov

The Jackson Laboratory, Bar Harbor, ME 04609, USA

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Abstract. "S" (survivor) mutants were produced in mice for genetic analysis of host resistance to metastatic cancers. S-mutants S-27 and S-31 resist transplantation of lymphoma EL4 of parental C57BL/6J (B6) mice while they accept parental skin grafts. Mutant S-27 also resists formation of spontaneous metastases from intradermally growing EL4 tumor into lymph nodes; mutant S-31 is highly susceptible to EL4 metastases. Another mutant, $H-2^{bm26}$ (bm26), resists EL4 and rejects B6 skin grafts. Major histocompatibility complex (MHC) class I and class II gene expression was compared in these mutants and normal B6 mice. All three mutants tested, S-27, S-31, and bm26, expressed a low amount of $K^b$ mRNA in organ-specific fashion. Mutants bm26 and S-31 expressed a low amount of $Ab^b$ mRNA and of $A^b$ antigen on their spleen cells. Some oligonucleotide probes designed to hybridize to the second exon of the class II MHC gene $Ab^b$ did not hybridize with DNA from all three mutants. These findings suggest extensive sequence alterations in the $Ab^b$ gene in mutants S-27, S-31, and bm26; they also suggest a major role of MHC in the control of host resistance to spontaneous metastases of the EL4 tumor.

Materials and methods

Introduction

Recently mouse mutants were found which accept parental strain skin grafts but resist a syngeneic tumor transplantation; in some of them resistance to tumor metastases is also affected (Egorov and Egorov 1988; Egorov et al. 1990; Egorov et al. 1992). They were designated S-mutants; some S-mutants are linked to $H-2$, which is the mouse major histocompatibility complex (MHC). Since aberrant expression on tumor cells of MHC class I antigens has been reported to play a role in malignancy and metastasis (Gopas et al. 1989 and references therein), one possibility is that these mutation(s) altered the expression and/or the primary structure of some MHC molecules. This paper describes tests of MHC gene expression in a group of $H-2$-linked S-mutants and the results obtained.

Probes. Oligonucleotides were synthesized on an automated solid-phase synthesizer (Applied Biosystems, Foster City, CA) by the standard phosphoramidite coupling method and are described in the Figure 1 legend. The class I MHC cDNA probes were pH-2IIa and pH-2III (Steinmetz et al. 1981). The class II gene $Ab^e$ cDNA-derived probe was p2894 (Robinson et al. 1983). The human $\alpha$-tubulin cDNA fragment or $\beta$-m-eDNA fragment (Gussow et al. 1987) were used as reference probes for quantitation of individual gene transcripts.

Slot blots. All procedures were essentially as described by Blouin and co-workers (1990). Briefly, nucleic acid concentrations were assessed by measuring their optical density (OD) at 260 nm. The samples were heated to 55 °C for 5 min and then their OD at 260 nm was measured again for accuracy. The samples were blotted on precoat nitrocellulose filters (BRL Life Technologies, Gaithersburg, MD) and the whole filters were rinsed in 2 × standard sodium citrate (SSC) for 5 min and baked at 80 °C for 2 h.
Flow cytometry analysis. The RNA samples were denatured in 1 × MOPS buffer containing 2.2 M formaldehyde and 50% formamide. The samples were heated to 65 °C for 5 min and then kept on ice for 2 min. The samples were loaded into 1% Agarose gel in 1 × MOPS buffer containing 2.2 M formaldehyde. The running buffer was 1 × MOPS buffer and the running time was 3 h at 100 V. The RNA was transferred and fixed to nitrocellulose paper (Scheicher and Schuell, Keene, NH) with 20 × SSC using the standard method (Sambrook et al. 1989). The filter was then dried and baked as for slot blots.

Hybridization procedures. Standard procedures (Sambrook et al. 1989) were used with some modifications. For oligonucleotide probes, the filters were prehybridized in 6 × SSC, 5 × Denhardt’s solution [1 × Denhardt’s = 0.02% Ficol 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin (BSA)] containing boiled sonicated salmon sperm DNA at 0.1 mg/ml for 2–4 h at incubation temperature (Ti) specific for each oligonucleotide. The filters were hybridized overnight at Ti in the same solution in the presence of Dextran sulfate at 100 mg/ml and the endlabeled oligonucleotide probe. The probes were endlabeled using gamma-32P-ATP (NEN Research Products, Wilmington, DE). The filters were washed using 3 M Me4NC1 method (Wood et al. 1985) to control the stringency of hybridization. For cDNA-derived probes, the filters were prehybridized in the Church hybridization buffer [40 mM Na2HPO4, pH 7.2, 1 mM ethylenediaminetetraacetate (EDTA), 7% sodium dodecyl sulfate (SDS), 1% BSA] at 65 °C for 2–12 h. The filters were hybridized overnight at 65 °C in the same solution in the presence of the labeled probe. The probes were labeled by random priming kit (Pharmacia) using α-32P-dCTP (NEN Research Products, Wilmington, DE). The filters were washed first with 1° Church wash buffer (0.5% BSA, 1 mM EDTA, 40 mM Na2HPO4, 5% SDS) at 65 °C for 30 min and then washed again with 2° Church wash buffer (1 mM EDTA, 40 mM Na2HPO4, 1% SDS) at 65 °C for 30 min. For rehybridization of the filters, stripping labeled probe technique (Meinkoth and Wahl 1984) was used.

Authoradiography. Autoradiograms were obtained by exposure of X-OMAT AR films (Eastman Kodak, Rochester, NY) with intensifying screens at −70 °C for 24 h.

Flow cytometry analysis. Standard test procedures were utilized. Briefly, 1 × 10⁶ cells were placed in tubes and washed with Hank’s balanced salt solution (HBSS) containing 0.2% BSA and 0.1% sodium azide. The cells were then incubated with 50 μl of properly diluted biotinilated monoclonal antibody (mAb) or with medium alone for 30 min at 4 °C. The cells were washed three times with the same medium and then incubated with a saturating concentration of streptavidin-PE conjugate for 30 min at 4 °C. The labeled cells were analyzed for the expression of MHC antigens on a FACSCAN flow cytometer (Beckton Dickinson, Mountain View, CA).

Results

For the analysis of class I and class II MHC mRNA expression in S-mutants, gene-specific oligonucleotide probes (Mellor 1987) were synthesized. These oligonucleotides were tested for specificity of hybridization in slot blot tests with DNA prepared from a panel of H-2 recombinant and mutant strains. K° oligo (Fig. 1) and D° oligo (data not shown) hybridized normally with DNA samples from all mutants and recombinants. Oligo Abb° [second exon of the Abb° gene, 3839–3859 base pair (bp)], (Larhammar et al. 1983); 38–58 bp (Choi et al. 1983)] did not hybridize with DNA from mutants S-27 and S-31 (Fig. 1). This result indicates that nucleotide sequence alteration(s) is present in the Abb° gene of both mutants. To determine the extent of the sequence alteration, five additional oligonucleotide probes were synthesized and tested (Fig. 1). They detected extensive sequence alterations in both S-27 and S-31 mutants which covered a large portion of the second exon (encoding for the first domain of the polypeptide chain); however, normal sequences were found in intron 1 and intron 2 of the Abb° gene in the mutants. Sequence alterations were detected also in the second exon of the Abb° gene in mutant bm26. One of these five oligonucleotides, bm12NR, was designed to hybridize to the normal Abb° sequence containing the site

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**Fig. 1.** Slot blot hybridization of spleen DNA from mutants to gene-specific oligonucleotide probes. B6, C57BL/6J strain DNA (normal H-2 haplotype). Synthetic oligonucleotides used: K° - 5'-GCCAGAG-ATCACCTGAAATAGT-3' (designed to detect K° gene transcript, spans 353–373 bp), 5673 - 5'-TCTGTATTTGCCAGGCACTGG-3' (first intron of the Abb° gene, 3573–3593 bp), 3809 - 5'-TGAGGACGAGCCCGTGCCGCCGCC-3' (second intron of the Abb° gene, 3573–3593 bp), 3809 - 5'-TGACCGCCCGTGCCCGACCCCGGG-3' (first intron-second exon of the Abb° gene, 3809–3829 bp). Abb° - 5'-CGACTCGCATTGAACCTGGTGTA-3' (designed to detect Abb° gene transcript, second exon of the Abb° gene, 3839–3859 bp), bm12NR - 5'-GAGATCCTGGAGCGAACGCGG-3' (second exon of the Abb° gene, 4010–4030 bp, spans the site of the bm12 mutation), 5673 - 5'-TCTGTATTTGCCAGGACCCCGGGG-3' (second intron of the Abb° gene, 5673–5693 bp). Filters were washed and rehybridized to a reference probe (human α-tubulin cDNA-derived probe). Oligonucleotides K° and Abb° were also tested with a panel of intra-H-2 recombinants. K° probe hybridized to B10.A(5R) (K°Abb°D°) and B10.MBR (K°Abb°D°), but did not hybridize to B10.A(2R) (K°Abb°D°) and B10.A (K°Abb°D°) spleen DNA; Abb° probe hybridized to B10.A(5R) but did not hybridize to B10.MBR, B10.A(2R), or B10.A spleen DNA (data not shown). Nucleotide sequence of K° gene (Kuhner and Goodenow 1989); nucleotide sequence of Abb° gene (Larhammar et al. 1983).