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Immunology

Allelic forms of β_2-microglobulin in the mouse

(major histocompatibility complex/immunoglobulin genetics/H-2 restriction)

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ABSTRACT Spleen cells from BALB/c and C57BL/6 mice were cultured separately or together, and the biosynthetically labeled supernates were examined by two-dimensional polyacrylamide gel electrophoresis. Although there were no major labeled proteins in the mixed group that were not present in the separate cultures, there was a major low-molecular-weight protein that differed in charge in the two strains. This protein was identified as β_2-microglobulin; it could be labeled with ^{125}I on the cell surface by using the lactoperoxidase technique, was noncovalently attached to the H-2K molecule, and had the expected size and charge when compared with human β_2-microglobulin. Both acidic and basic forms were present in (BALB/c × C57BL/6) F_1 hybrids, suggesting codominant expression, although allelic exclusion was not ruled out. Either parental form could combine with one parental form of the H-2K molecule. The β_2-microglobulin gene does not appear to be closely linked to either the H-2 or the immunoglobulin heavy-chain complexes. It is proposed that β_2-microglobulin is an "effector subunit" of histocompatibility antigens and that its physiological role is to interact with a specific killing structure on the surface of cytolytic T lymphocytes and thereby initiate cell destruction.

The major histocompatibility complex has three main classes of loci (1). Class I loci code for M_r 45,000 polypeptides, which are expressed on the surface of virtually all nucleated cells. Class II loci code for a family of cell surface proteins of $M_r \approx 30,000$, which are present on B lymphocytes, macrophages, and some T lymphocytes. Class III loci code for molecules that are components of the complement system.

Class I loci probably arose by gene duplication and show extensive genetic polymorphism (1). The M_r 45,000 products are attached to the plasma membrane by a hydrophobic segment, and their extracellular portion is noncovalently associated with β_2-microglobulin (β_2m), a highly conserved water-soluble protein of M_r 12,000, that shows significant homology to the C_H3 domain of immunoglobulin γ chains (2).

The polymorphism of the H-2 heavy chains seems to have functional significance in that recognition of both self-H-2 and foreign antigen seems to be essential for T-lymphocyte activation (1). However, the significance of the association of a highly polymorphic heavy chain with an invariant immunoglobulin-like light chain is unknown.

Although the genetics of the M_r 45,000 heavy chain have been extensively investigated, the high degree of conservation of β_2m has hindered the study of its inheritance. Analysis of hybrids between human and mouse cells has established that the gene for human β_2m is on chromosome 15 while that for the HLA complex is on chromosome 6 (3). The location of the β_2m gene in other species is not known.

In this paper, we report the existence of allelic forms of β_2m and present preliminary analysis of its inheritance and a hypothesis concerning its function.

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MATERIALS AND METHODS

Cell Culture. Spleen cells from 3-month-old female C57BL/6 and BALB/c mice were cultured in RPMI 1640 medium containing 10% fetal calf serum at a density of 10,000,000 cells per ml. After 3 days, the cells were washed and resuspended in 1 ml of methionine-free RPMI 1640 containing 10% dialyzed fetal calf serum and 300 μCi of [^{35}S]methionine (800 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels; Amersham). After a further 4 hr at 37°C, the cells were removed by centrifugation and the supernates were analyzed.

Cell Surface Radioiodination. This was carried out exactly as described (4). Cells were solubilized in 0.5 ml of 0.5% Triton X-100 in phosphate-buffered saline [164 mM Na^+/4 mM K^+/132 mM Cl^-/20 mM $(PO_4)^{3-}$, pH 7.4], and the nuclei were removed by centrifugation.

Immunoprecipitation. Monoclonal anti-H-2K^k was from clone 11-4 (5). The cell lysate was diluted to 2.0 ml with 20 mM sodium phosphate (pH 7.4) containing 0.5% Triton X-100 (6), and 5 μg of antibody was added. After 1 hr at 4°C, 50 μl of a 10% (vol/vol) suspension of heat-killed formalin-fixed *Staphylococcus aureus* was added. Staphylococci were washed twice with 20 mM phosphate (pH 7.4) containing 0.5% Triton X-100.

Two-dimensional polyacrylamide gel electrophoresis was carried out as described by O'Farrell *et al.* (7). Gels were aligned by reference to nonradioactive stained marker proteins. Human β_2m was purified from human urine by the method of Cejka and Poulik (8).

RESULTS

Allelic Forms of a Low-Molecular-Weight Protein in Spleen Cell Supernates. Spleen cells from BALB/c and C57BL/6 mice were cultured separately or together, and the biosynthetically labeled secreted products were analyzed by gel electrophoresis (Fig. 1). As might be expected, the major protein secreted was IgM, the identity of which was confirmed by specific immunoprecipitation (data not shown; see ref. 9). Although the experiment was designed to identify lymphokine-like proteins secreted as a result of allogeneic stimulation when lymphocytes from the two strains were cultured together, no proteins could be identified that were not present in one or the other supernate when the cells were cultured alone.

In addition to immunoglobulin, a heavily labeled low molecular weight protein was observed (Fig. 1). Although this protein was present in all cultures, the form found in C57BL supernates was distinctly more basic and slightly smaller than that found in BALB/c supernates. When the cells from the two strains were cultured together, both forms were found. Cultures of (C57BL × BALB/c) F_1 hybrids showed a pattern identical to that of a mixture of the parental cells (data not shown).

The low molecular weight protein was water soluble, as

Abbreviation: β_2m, β_2-microglobulin.

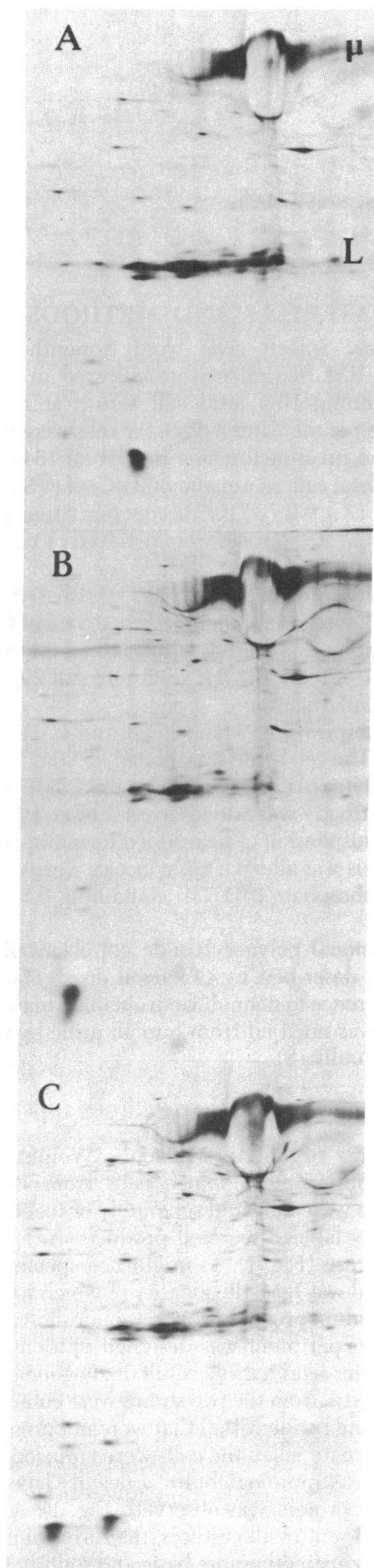

FIG. 1. Gel electrophoresis of ^{35}S-labeled proteins in supernates of cultured spleen cells. Ten million spleen cells of BALB/c (*A*) or C57BL/6 (*B*) mice or a mixture of 5,000,000 of each (*C*) were cultured for 3 days and then washed and recultured for a further 4 hr in the presence of [^{35}S]methionine. Supernates were made 9 M in urea and analyzed. Acidic proteins lie to the right. μ, Immunoglobulin μ; L, light chains. The concentration of acrylamide in the second dimension was 13%. Distortions are due to overloading by fetal calf serum.

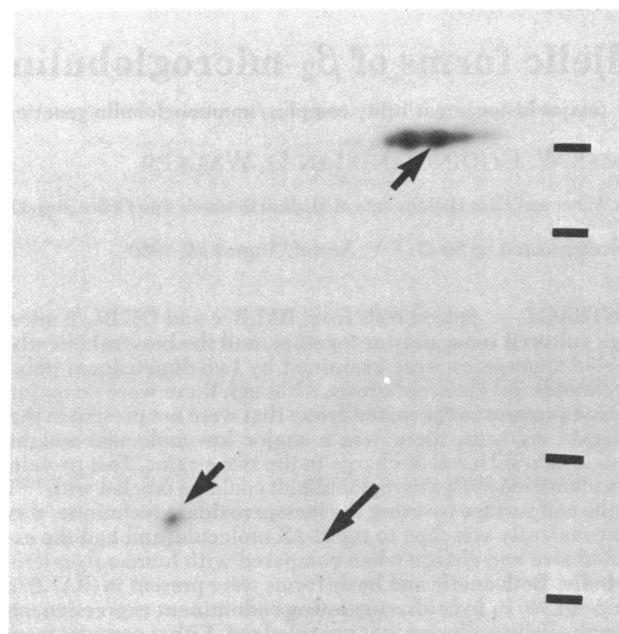

FIG. 2. Gel electrophoresis of ^{125}I-labeled proteins precipitated by anti-H-2K^k antibody from detergent lysates of surface-radioiodinated AKR mouse spleen cells. Horizontal bars indicate molecular weight standards [ovalbumin (M_r = 43,000), lactate dehydrogenase (M_r = 30,000), soybean trypsin inhibitor (M_r = 20,000), and lysozyme (M_r = 14,000)]. The H-2K molecule is indicated by the short arrow pointing up, and murine β_2m is indicated by the short arrow pointing down. The long arrow pointing down indicates stained nonradioactive human β_2m. The second dimension was a linear gradient of 10–15% acrylamide.

shown by the fact that it remained in solution after centrifugation at 100,000 × *g* for 20 min in a Beckman Airfuge in the absence of detergent.

Identification of the Protein as β_2m. Cultured spleen cells secrete free β_2m (10), and the size and charge of the low molecular weight polypeptide seen in Fig. 1 were consistent with this identity. Because the H-2K molecule is noncovalently associated with β_2m (11), we reasoned that, if the protein were indeed β_2m, it should be specifically immunoprecipitated by antibodies to H-2K.

Cells from AKR mice were surface radioiodinated with ^{125}I, their membranes were solubilized in Triton X-100, and the H-2K^k molecule was immunoprecipitated and analyzed by gel electrophoresis (Fig. 2). As expected, the H-2K heavy chain had an apparent M_r of 45,000 and was associated with a polypeptide of apparent M_r 15,000 that was somewhat more basic and slightly larger than human β_2m. These differences are consistent with the known properties of mouse and human β_2m (6, 12). Taken together, the data positively identify the M_r 15,000 polypeptide seen in Fig. 2 as β_2m.

Because the gene for human β_2m is on a different chromosome from that for the HLA complex (3) and there may be a similar conservation of syntenic gene groups in mice and humans (13), we thought it likely that the gene for β_2m in the mouse would not be linked to H-2. Thus, we expected that BALB/c.H-2^k mice and B10.BR mice (which are also H-2^k) would differ in their β_2m alleles. Fig. 3 shows that this was the case.

Spleen cells from BALB/c.H-2^k and B10.BR mice were surface radioiodinated, and the H-2K^k molecule was isolated by immunoprecipitation and analyzed by gel electrophoresis. The H-2K^k molecules from the two strains migrated in identical positions, but the β_2m molecule from BALB/c.H-2^k mice was

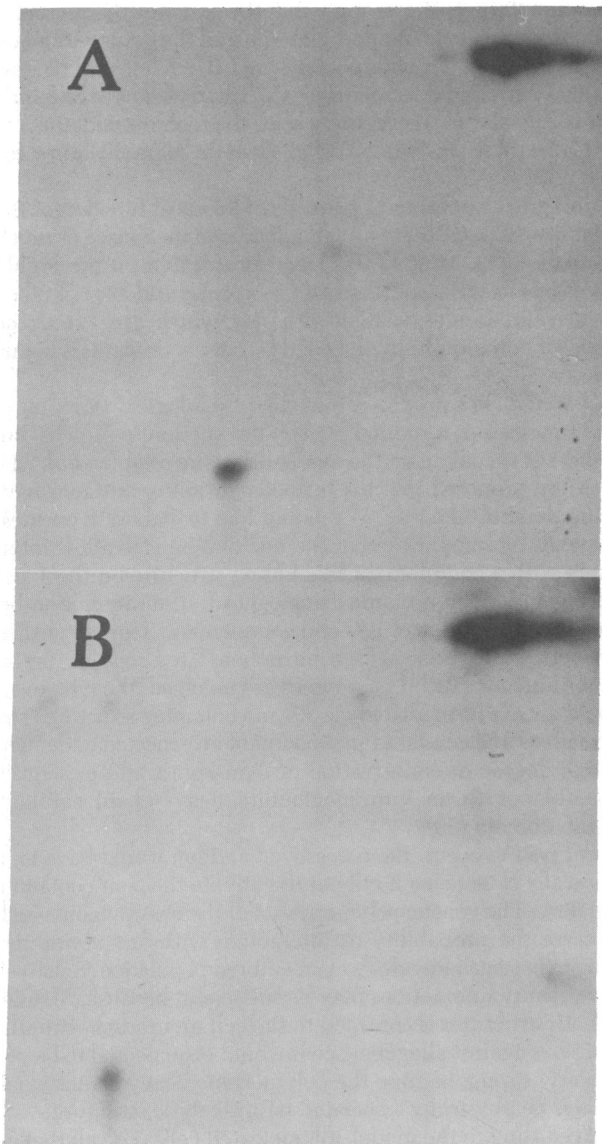

FIG. 3. Gel electrophoresis of ^{125}I-labeled H-2K and β_2m precipitated from spleen cells of BALB/c.H-2^k (*A*) or B10.BR (*B*) mice. The concentration of acrylamide in the second dimension was 15%.

more acidic than that from B10.BR mice. CBA and AKR mice (H-2^k) also had the acidic form of β_2m, and the H-$2K^k$ molecule from (C57BL/6 × CBA) F_1 mice was associated with both forms (data not shown).

The slight charge heterogeneity of radioiodinated β_2m probably reflects a decrease in the pK of the phenolic hydroxyl of tyrosine by the ortho substitution of iodine for hydrogen (14–16).

Distribution of β_2m Alleles in Different Strains. To extend the genetic analysis of β_2m to strains that do not possess the H-$2K^k$ molecule, we examined the whole extract of detergent-solubilized surface-radioiodinated spleen cells by gel electrophoresis. Parallel analyses of immunoprecipitates showed that H-$2K^k$ and β_2m could be identified without immunoprecipitation (Fig. 4). Similar identifications are possible for many other lymphocyte surface proteins (17, 18).

A preliminary strain survey showed that all strains with a C57BL genetic background had the basic form of β_2m, regardless of their H-2 type or immunoglobulin heavy-chain haplotype, and that all other strains had the acidic allele (Table 1). These results argue against close linkage of the β_2m gene to H-2 or the immunoglobulin heavy-chain complex.

Table 1. Strain distribution of β_2m alleles

Strain	β_2m	H-2	Igh-C
BALB/cWehi F?+F13fF19	a	d	a
BALB/c.Ig-1^b N20F2fF8	a	d	b
BALB/c.H-2^k N12F?+N5F3fF1	a	k	a
NZB/B1Wehi F42+F48fF10	a	d	n
CBA/CaHWehi F?+F16fF26	a	k	j
AKR/JWehi F128+F1fF10	a	k	d
C57BL/6JWehi F105+F1fF17	b	b	b
C57BL/6.Ig-1^n N20F4fF3	b	b	n
B10.A Non Pedigree +F6fF7	b	a	b
B10.BRSgSn N9F32+F2fF11	b	k	b
B10.D2/nSn N?F43+F3fF9	b	d	b

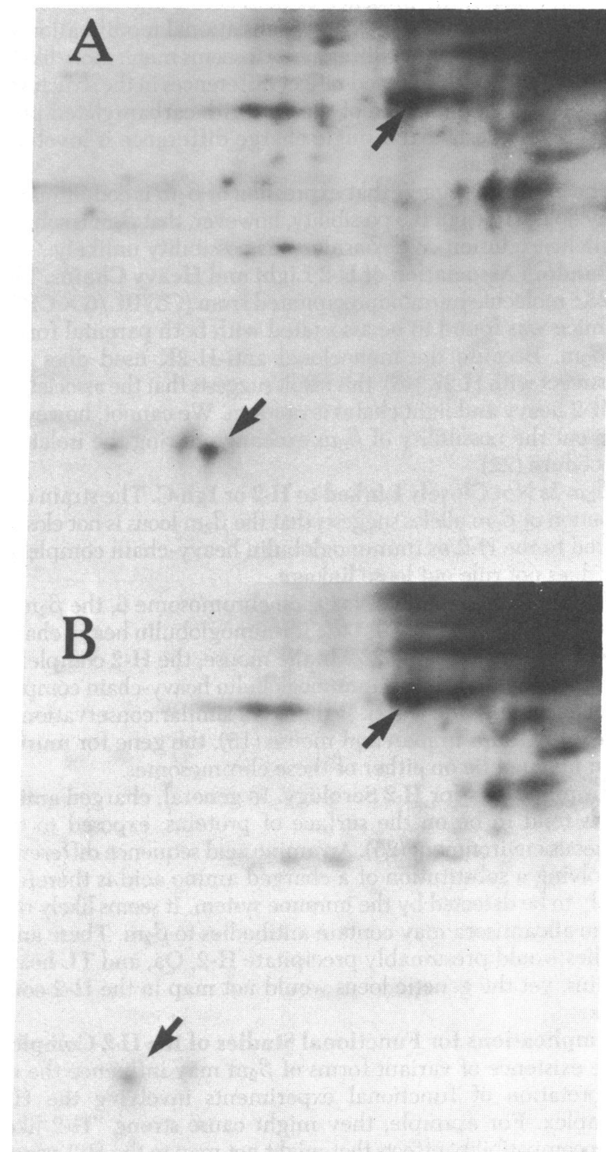

FIG. 4. Gel electrophoresis of total ^{125}I-labeled cell surface proteins from BALB/c.H-2^k (*A*) or B10.A (*B*) mice. Positions of H-2K (upward arrows) and β_2m (downward arrows) were determined by parallel immunoprecipitation and gel electrophoresis (data not shown). The concentration of acrylamide in the second dimension was 15%.

DISCUSSION

Allelic Forms of β_2m. We have presented evidence for a charge difference between the β_2m synthesized by BALB/c mice and that synthesized by C57BL/6 mice. The evidence that the protein is β_2m rests on its noncovalent association with the H-2K^k heavy chain, its apparent molecular weight, and its mobility relative to human β_2m. Although we did not use specific antisera to β_2m, the association between major histocompatibility complex products and β_2m is extensively documented (6, 11, 12, 15, 19). Moreover, the serological specificity against purified mouse β_2m of the monoclonal anti-H-2K^k antibody is likely to be much greater than that of conventional heterologous antisera.

The charge difference could reflect differences in the DNA sequence coding for β_2m or, possibly, post-translational modification (20). Phosphorylation of β_2m is unlikely to account for the charge difference, because β_2m is an extracellular peripheral membrane protein. Carbohydrate differences, such as sialic acid heterogeneity, are unlikely, because β_2m is not glycosylated (2). Finally, the presence of both acidic and basic forms in F_1 hybrids argues against post-translational modification as an explanation of charge differences. It seems much more likely that the differences observed reflect differences in the structural gene for β_2m. Calibration of the gels with carbamylated proteins (21) suggests that a single charge difference is involved (unpublished data).

The data also suggest that expression of β_2m is codominant. They do not rule out the possibility, however, that β_2m is subject to allelic exclusion. We consider this possibility unlikely.

Random Association of H-2 Light and Heavy Chains. The H-2K^k molecule immunoprecipitated from (C57BL/6 × CBA) F_1 mice was found to be associated with both parental forms of β_2m. Because the monoclonal anti-H-2K used does not crossreact with H-2K^b (5), this result suggests that the association of H-2 heavy and light chains is random. We cannot, however, rule out the possibility of β_2m exchange during the isolation procedure (22).

β_2m Is Not Closely Linked to H-2 or Igh-C. The strain distribution of β_2m alleles suggests that the β_2m locus is not closely linked to the H-2 or immunoglobulin heavy-chain complexes but does not rule out loose linkage.

In man, the HLA complex is on chromosome 6, the β_2m is on chromosome 15 (3), and the immunoglobulin heavy chains are on chromosome 14 (23). In the mouse, the H-2 complex is chromosome 17 and the immunoglobulin heavy-chain complex is on chromosome 12 (24). If there is a similar conservation of syntenic groups in man and mouse (13), the gene for murine β_2m may not lie on either of these chromosomes.

Implications for H-2 Serology. In general, charged amino acids tend to be on the surface of proteins, exposed to the aqueous environment (25). An amino acid sequence difference involving a substitution of a charged amino acid is therefore likely to be detected by the immune system. It seems likely that some alloantisera may contain antibodies to β_2m. These antibodies would presumably precipitate H-2, Qa, and TL heavy chains, yet the genetic locus would not map in the H-2 complex.

Implications for Functional Studies of the H-2 Complex. The existence of variant forms of β_2m may influence the interpretation of functional experiments involving the H-2 complex. For example, they might cause strong "H-2-like" histocompatibility effects that might not map to the H-2 region. The H-2 heavy chains are poorly recognized by alloantisera in the absence of β_2m (26). It therefore seems likely that the conformation of β_2m influences the conformation of the H-2 heavy chains and might cause a form of "H-2 restriction" that did not map to the H-2 complex.

Nomenclature. We propose that the gene for β_2-microglobulin be named beta-2-microglobulin and the gene symbol be designated *b2m*. The more-acidic BALB/c allele will be designated *b2m^a*, and the more-basic C57BL/6 allele will be *b2m^b*. This nomenclature is consistent with the recommendations of the Committee on Standardized Genetic Nomenclature for Mice (27).

Biological Function of β_2m. The biological function of the major histocompatibility antigens has been the source of much speculation (1). Most of this has concentrated on the highly polymorphic nature of the class I molecules and has not taken into account their association with β_2m, which, apart from the restricted polymorphism suggested by our experiments is highly conserved.

Klein (28) has proposed that the physiological function of class I molecules is to mediate cell destruction by T cells, but he did not speculate on the mechanism. Barnstable *et al.* (29) have also proposed that the histocompatibility antigens have a complement-like role. We would like to elaborate on these proposals by suggesting that the role of β_2m is to allow interaction with a complement-like killing structure on the T cell (perhaps Ly 2) in a manner analogous to the interaction between the Fc portion of IgG and complement. Thus, regardless of whether allogeneic cells, trinitrophenylated cells, viruses, or minor histocompatibility antigens are involved, the lytic event would always be mediated via a β_2m-containing structure. This specialized and constant function might be expected to demand a high degree of conservation of β_2m and might explain its resemblance to an immunoglobulin heavy-chain constant-region domain (30).

For lysis to occur, the recognized antigen would have to be physically close (even if only transiently) to the β_2m-containing structure. The genetic polymorphism of the heavy chains would increase the probability of interaction with any given viral antigen in some individuals of an outbred population. Relatively low-affinity interactions may be sufficient, because diffusion of both structures is confined to the cell membrane. Immune reactions against allogeneic cells would be expected to be particularly strong, because the polymorphic heavy chains would always be physically associated with the lytic structure.

According to the model, all nucleated cells (i.e., all that are capable of supporting viral replication) have an inbuilt mechanism for their own destruction via interaction with T cells. Although this would not be in the interest of the individual cell, it would be of great survival value to the whole animal.

Note Added in Proof. After this paper was submitted, we learned of similar findings by Michaelson *et al.* (31). Our data and nomenclature are in complete agreement. We have now completed a strain survey of β_2m alleles. Strains C57BL/6, C57BL/10, C57BR, C57L, CXBE, CXBG, CXBI, CXBJ, and CXBK were b2m^b. Strains C58, RF, CXBD, CXBH and B10.C(28NX) were b2m^a. These results suggest close linkage to H-3 and Ly-4. It is possible that β_2m, H-3, and Ly-4 are identical (32).

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