HLA-A2 MUTANTS IMMUNOSELECTED IN VITRO

Definition of Residues Contributing to an HLA-A2-specific Serological Determinant*

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The major histocompatibility complex (MHC)\(^1\) controls the expression of a variety of highly polymorphic cell surface antigens that play important roles in various aspects of the immune response (1-3). The class I molecules (human HLA-A, -B, and -C, and murine H-2K,D and L) are present on all nucleated cells and carry foreign determinants important in immune recognition by both alloantibodies and alloreactive cytotoxic T lymphocytes (CTL), and the self-determinants important in immune recognition of virally infected cells by CTL.

HLA-A, -B, and -C antigens are intrinsic membrane glycoproteins composed of a polymorphic, MHC-encoded heavy chain of 44,000 mol wt, noncovalently associated with an invariant, non-MHC-encoded light chain of 12,000 mol wt, \(\beta_2\) microglobulin (\(\beta_2m\)) (4). Primary structural studies have suggested that the heavy chain consists of three extracellular domains of \(\sim 90\) amino acids each. The two amino terminal domains are polymorphic, whereas the third domain is relatively nonpolymorphic. Both \(\beta_2m\) and the third extracellular domain display sequence homology with immunoglobulin-constant region domains.

Multiple amino acid substitutions distinguish even closely related allelic products; however, comparisons of a limited number amino acid sequences have indicated the presence of three or four more or less discrete segments of variability (5). It is clear from studies of both serologic and cellular reactivities that multiple allospecific recognition determinants exist (1, 6-8). However, the locations of and relationships among these determinants have not been defined.

The HLA-A2-specific mouse monoclonal antibody BB7.2 (9) and complement has been used to immunoselect variant clones of the B lymphoblastoid cell line T5-1 (HLA-A1, -A2, -B8, and -B27).\(^2\) Three major classes of variant clones have been distinguished; members of two classes display little or no expression of cell surface HLA-A2 molecules. In one case, this results from an apparent failure to synthesize HLA-A2 heavy chains, and in the other case, although structurally altered HLA-A2...
Heavy chains are synthesized, they associate with β2m inefficiently, if at all. Previous studies have demonstrated that mutant HLA-A2 heavy chains that fail to associate with β2m neither mature to the cell surface nor express alloantigenic determinants (10).

Members of a third class appear to express normal amounts of cell-surface HLA-A2 molecules that display reduced reactivity with the selecting antibody, but normal or near-normal reactivities with some other HLA-A2-specific monoclonal antibodies and human alloantisera. Such properties indicated their potential utility in defining the BB7.2 epitope, as well as in probing the relationships among various class I antigen serologic and cellular recognition determinants.

Structural studies of the HLA-A2 heavy chains derived from two of these variants, 8.18.1 and 8.21.1, are described in the present study. These variants are distinguishable both from TS-1 and from each other by their relative reactivities with the selecting antibody BB7.2, as well as with a second HLA-A2-specific monoclonal antibody, PA2.1. In addition, HLA-A2 heavy chains synthesized by 8.18.1 are the equivalent of a single charge more basic than those of TS-1, whereas those synthesized by 8.21.1 are the equivalent of a single charge more acidic. Comparative tryptic peptide analyses presented here indicate that these heavy chains carry distinct mutations in the same peptide. We conclude that residues within this short segment of the polypeptide contribute to an HLA-A2-specific serological determinant.

Materials and Methods

Cell Lines and Metabolic Labeling. The human B lymphoblastoid cell line T5-1 (HLA-A1, -A2, -B8, -B27, and -Cw1) and the HLA-A2 variant clones 8.18.1 and 8.21.1 derived from T5-1 by mutagenesis followed by immunoselection were cultured as described (10). [4,5-3H]Lysine and leucine, [2,3-3H]proline and alanine, [G-3H]serine, the respective U-14C amino acids, [methyl-3H]methionine, [35S]methionine, and [35S]cysteine were obtained from either New England Nuclear, Boston, MA or ICN Chemical and Radioisotope Div., ICN Pharmaceuticals, Inc., Irvine, CA. Conditions for metabolic labeling were as described (11).

Purification of Radiolabeled HLA-A2 Heavy Chains. This procedure has been described in detail elsewhere (11). Briefly, detergent lysates were prepared from labeled cells and immunoprecipitated with the HLA-A2-specific mouse monoclonal antibody PA2.1 (12). After denaturation, immunoprecipitated species were then subjected to a second round of immunoprecipitation, this time using a rabbit anti-HLA heavy chain serum (13). The resulting HLA heavy chain preparations were of >95% purity, as assessed sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography. Yields of HLA-A2 heavy chain radioactivity as a percentage of total incorporation into cells were typically 0.05% for TS-1, half that for 8.18.1, and half again for 8.21.1, because of reduced reactivities with the PA2.1 antibody.

Peptide Mapping. 3H-, 14C-, or 35S-labeled HLA-A2 heavy chain preparations were combined, reduced and alkylated, and digested with trypsin as described (11). Peptide mapping was by reverse-phase high-pressure liquid chromatography (HPLC) on a 4-mm × 25-cm Zorbax C-8 column (Dupont Instruments, Wilmington, DE), using a 0–30% concave gradient of acetonitrile in 10 mM ammonium acetate (pH 6.5) as described (11). Radioactivity was determined by liquid scintillation counting.

Microsequence Analysis. This was performed on a Beckman liquid-phase sequencer 890C (Beckman Instruments, Inc., Fullerton, CA) as described (11, 14).

Results

Strategy. To assess the relatedness of the HLA-A2 heavy chains synthesized by the parent cell line T5-1 and its immunoselected variants, cells were metabolically labeled
with either $^3$H or $^{14}$C amino acids, and purified heavy chains were compared by double-label tryptic peptide analysis. Crucial to such an analysis is the ability to compare most, if not all, tryptic peptides in the molecule. 90% of the amino acid sequence of the 271 residue papain solubilized HLA-A2 molecule (5), as well as 32 residues of the carboxy-terminal, intracellular portion have been determined (15). Thus, most tryptic peptides can be predicted from the known sequence. Tentative assignments for the remaining residues based upon homology with HLA-A28 (96% homologous) or with HLA-B7 (86% homologous) when both HLA-A2 and -A28 are unassigned, predict 41 tryptic peptides. Although a combination of radioactive lysine and arginine should label all but the carboxy-terminal peptide, this approach could not be taken because of an inability to incorporate sufficient amounts of radiolabeled arginine. The alternative adopted involved metabolic labeling with five different amino acids, which would label overlapping sets of tryptic peptides encompassing virtually the entire molecule. The amino acids used, lysine (K), leucine (L), serine (S), proline (P), and alanine (A), were predicted to label 11, 16, 11, 11, and 17 peptides, respectively, covering 98% of the detergent solubilized molecule. These numbers must be regarded with caution pending definitive assignment at the remaining residues of the HLA-A2 sequence.

Previous work (11) has indicated that peptide mapping by reverse-phase HPLC is a sensitive method with which to compare most tryptic peptides in the molecule. Thus, in the present study, radiolabeled HLA-A2 heavy chains purified from parent and variant cell lines were combined, reduced and alkylated, and digested with trypsin, and the resulting fragments were resolved and compared by HPLC.

8.18.1. Lysine-, leucine-, serine-, proline-, and alanine-containing tryptic peptides derived from T5-1 and 8.18.1 are compared in Figs. 1-5, respectively. For the purpose of discussion, individual peaks will be referred to based upon the amino acid label.
Fig. 2. Leucine-labeled tryptic peptides: $[^{14}C]$leucine-labeled T5-1 (—) vs. $[^{3}H]$leucine-labeled 8.18.1 (⋯). Note that the acetonitrile gradient used is different from that in other comparisons, as described (11).

Fig. 3. Serine-labeled tryptic peptides: $[^{14}C]$serine-labeled T5-1 (—) vs. $[^{3}H]$serine-labeled 8.18.1 (⋯). Differences that are also evident in the control comparison (Fig. 6) are denoted by solid arrows, whereas those unique to the experimental comparison are denoted by open arrows. Note that the solid arrow at tube S141 marks a shoulder sometimes, but not always resolved from that at S138 (see Fig. 8, for example). The factors influencing this resolution are not cell line specific.
and peak fraction number of the peptide. Thus, the flow-through peak in the serine map would be referred to as peak S6. It is immediately apparent that these molecules are highly homologous. No differences can be observed in the lysine-, leucine-, proline-, or alanine-containing tryptic peptides. However, multiple differences in the $[^3]H$serine vs. $[^{14}C]$serine comparison are detected (Fig. 3).
Differences in the serine map can be grouped into two categories based upon a control comparison of \(^3\text{H}\)serine vs. \(^{14}\text{C}\)serine-labeled T5-1 HLA-A2, which is presented in Fig. 6. Thus, differences in Fig. 3 peaks S79, S82, S122, S130, and the shoulder at S141 (solid arrows) are also evident in the control comparison, whereas those in peaks S126 and S135 (open arrows) are not. Those differences present in the control comparison have been shown previously to result from the metabolic conversion of radiolabeled serine to glycine with preferential loss of \(^3\text{H}\) label during the interconversion (11). This is the only interconversion that has been detected.

Relative to the control comparison, the Fig. 3 comparison differs by the loss of \(^3\text{H}\) radioactivity at peak S126 and the gain of \(^3\text{H}\) radioactivity at peak S135. This suggested the possibility that T5-1 peptide S126 was altered in 8.18.1, and hence eluted later in the peptide profile, at S135. This interpretation was confirmed and the difference peptide was identified by the following experiments. Peptide S126 was isolated from \(^3\text{H}\)serine-labeled T5-1 HLA-A2 and subjected to 12 cycles of automated Edman degradation. Radioactivity was released at cycle 8 (Fig. 7A). Hydrolysis of the peptide followed by analysis by thin-layer chromatography indicated that virtually all of the radioactivity in this peptide migrated as serine and not glycine; thus, it could be concluded that S126 had a serine residue at position 8. Sequence analysis of peak S135 from double-label comparisons of \(^3\text{H}\)serine-labeled 8.18.1 and \(^{14}\text{C}\)serine-labeled T5-1 HLA-A2 yielded both \(^\text{3H}\) and \(^\text{14C}\) radioactivity at cycle 1, and \(^3\text{H}\) radioactivity at cycle 8 (Figs. 7B and C). We interpret this as a mixture of a peptide common to both cell lines with the 8.18.1 counterpart of T5-1 peptide S126. A comparison of these partial sequences with those of all predicted serine-containing tryptic peptides (Table I) suggested that T5-1 peptide S126 spanned either residues 98–108 or 244–256 in the HLA-A2 sequence, and that the T5-1 and 8.18.1 common

![Fig. 6. Serine-labeled tryptic peptides: \(^{14}\text{C}\)serine-labeled T5-1 (---) vs. \(^3\text{H}\)serine-labeled T5-1 (-----). Differences are denoted by solid arrows. That at S141 is barely resolved from the peak at S138 in this instance, but has been resolved on other occasions.](image-url)
peptide in peak S135 spanned residues 132-144. Because the difference peptide was observed in neither the proline nor the alanine maps (Figs. 4 and 5, respectively), and because the peptide spanning residues 244-256 contains both of these residues (Table I), it was most likely that peptide S126 spanned residues 98-108 of the HLA-A2 heavy chain.

Because identification of the difference peptide rested on the determination of only a single amino acid, further partial sequence and compositional information was desirable for confirmation. The sequence of the T5-1 form of this peptide is predicted to be MYGCDVGSDWR; thus, it is unique in being the only tryptic peptide in the molecule that contains both methionine and cysteine. The presence of cysteine in the difference peptide was demonstrated by two lines of evidence. First, [3H]serine-labeled T5-1 HLA-A2 reduced and alkylated with iodoacetamide as usual was compared with [14C]serine-labeled T5-1 HLA-A2 reduced and alkylated with iodoacetamide as usual was compared with [3H]serine-labeled T5-1 HLA-2 reduced and alkylated with iodoacetic acid. A difference in such a comparison would identify an alkylated peptide, and this was indeed observed for peak S126. Furthermore, separate peptide maps of [35S]cysteine-labeled T5-1 HLA-A2 and 8.18.1 HLA-A2 were performed. A T5-1 peak at fraction 126 was absent in 8.18.1, and a new peak in 8.18.1 appeared at fraction 135. Sequence analysis...
of both peaks confirmed the presence of cysteine at position 4 in both parent and mutant (Figs. 7 D and E). A double-label comparison using [3H]methionine and [35S]methionine demonstrated a similar difference between the two cell lines. Thus, we conclude that a difference or differences in a single detectable peptide, that which spans residues 98–108 in the HLA-A2 heavy chain sequence, distinguishes 8.18.1 HLA-A2 from the wild-type product.

8.21.1. Because variant 8.21.1 has properties similar, but not identical, to those of 8.18.1, it was of interest to determine whether it carried a mutation in the same peptide. Thus [14C]serine- and [3H]serine-labeled HLA-A2 heavy chain preparations from T5-1 and 8.21.1 were compared by a similar analysis (Fig. 8). All of the late-eluting peptides are displaced by about three fractions relative to previous comparisons. The T5-1 glycine peptides at fractions 125, 133, and 144 (solid arrows) are thus identical to Fig. 3 T5-1 peptides S121 and S130 and the shoulder at fraction 141, and the T5-1 serine peptides at fractions 129, 137, and 141 are identical to Fig. 3 T5-1 peptides S126, S134, and S138. It is clear from this comparison that the T5-1 peptide at fraction 129 (open arrow), corresponding to S126 in Fig. 3, does not have a co-migrating 8.21.1-derived counterpart. This indicates that 8.21.1 is altered in the same peptide as 8.18.1. However, the two mutations in this peptide appeared to be nonidentical, because the 8.21.1 form of the peptide was not evident, eluting somewhat later than its T5-1 counterpart, as was the 8.18.1 form.

To clarify this difference between the two mutants, it was of value to identify the 8.21.1 form of the difference peptide. It might not be observed in the Fig. 8 comparison because of altered solubility properties, poor elution off the column, the loss of a serine residue at position 8, or co-elution with higher-yield peptides. Some of these possibilities could be tested by choosing a different amino acid for labeling the peptide; methionine was an optimal choice because it is a relatively rare amino acid that labels the corresponding T5-1 peptide. A comparison of [3H]methionine- with [35S]methionine-labeled peptides is presented in Fig. 9. The two cell lines clearly differ in that the T5-1 peptide at M126 has apparently shifted to M88 in 8.21.1 (open arrows). This

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**Table 1**

**Serine-labeled Tryptic Peptides**

| Residue number | Serine position(s) | Other residue(s) |
|----------------|-------------------|-----------------|
| 1-6           | 2, 4              | —               |
| 7-14          | 5, 7              | —               |
| 36-44         | 3, 7              | Alanine         |
| 83-97         | 6, 10             | Alanine, leucine|
| 98-108‡      | 8                 | —               |
| 132-144§     | 1                 | Alanine, lysine |
| 187-202       | 9                 | Alanine, leucine|
| 244-256       | 8                 | Alanine, proline|
| 274-309|| | 3, 4, 6 | Alanine, proline, leucine |
| 312-315       | 1, 2              |                 |
| 317-338       | 3, 5, 9, 10, 12, 16, 19 | Alanine, leucine |

* Data are derived from refs. 4, 5, and 15.
‡ M. S. Krangel, unpublished evidence for D102 and R108.
§ By homology with HLA-A28 and -B7.
|| By homology with HLA-B7.
Fig. 8. Serine-labeled tryptic peptides: $[^{14}C]$serine-labeled T5-1 (−) vs. $[^{3}H]$serine-labeled 8.21.1 (····). Differences also evident in the control comparison (Fig. 6) are denoted by solid arrows. That unique to the experimental comparison is denoted by an open arrow.

Fig. 9. Methionine-labeled tryptic peptides: $[^{3}H]$methionine-labeled T5-1 (−) vs. $[^{35}S]$methionine-labeled 8.21.1 (····). Differences are denoted by open arrows.

corresponds to a cluster of peaks in the serine map, within which the $[^{3}H]$serine-labeled 8.21.1 difference peptide would have gone undetected. We cannot, from this analysis, eliminate the possibility of a loss of serine from this peptide, however. Additional experiments comparing in parallel $[^{35}S]$cysteine-labeled peptides from T5-
1 and 8.21.1 yielded results consistent with those of the methionine-containing peptide comparison.

Discussion

HLA-A2 mutants have been obtained by immunoselection using the HLA-A2-specific mouse monoclonal antibody BB7.2 plus complement. Two mutants that synthesize HLA-A2 molecules with reduced reactivity with the selecting antibody and with structurally altered HLA-A2 heavy chains have been analyzed in detail. Using double-label tryptic peptide mapping, we demonstrate that these two mutants have distinct alterations in the same tryptic peptide in the molecule. Although it is possible that other alterations in these molecules exist but have not been detected by the present analysis, the isolation of two mutants with alterations in the same peptide suggests strongly that the detected changes play crucial roles in determining the serologic properties of these molecules.

Available evidence suggests that the mutations do not induce gross structural alterations in these molecules. Thus, although reactivities with both the selecting antibody and the HLA-A2-specific monoclonal antibody PA2.1 are diminished, reactivities with a polyclonal HLA-A2-specific human alloantiserum are largely unaffected, and no effect at all has been observed with respect to the binding of the HLA-A2 and -B specific monoclonal antibody MA2.1 (16) and the HLA-A, -B, and -C-specific monoclonal antibody W6/32. The determinants recognized by all such antibodies are highly sensitive to the conformation of the molecule. Furthermore, although the altered peptide is known to contain a cysteine involved in an intramolecular disulfide bond, the results of labelings with [35S]cysteine indicate that this residue has not changed in either mutant. It is therefore likely that a residue or residues in this peptide play a direct role in forming an HLA-A2-specific antigenic determinant.

Comparative primary structural analyses of three HLA molecules have indicated the presence of a few discrete segments that display relatively high variability, the most prominent of which span residues 65–80, 105–116, and 177–194. HLA-A2 and -A28 are highly cross-reactive, and only 10 amino acid differences have been detected between them (5). Five are in the first segment, two are in the second, and the remainder are highly conservative substitutions scattered throughout the molecule. The difference peptide defined in this study spans residues 98–108 and has the sequence MYGCDVGSDWR. HLA-A28 has an identical sequence, save for a W to G substitution at position 107. Because the monoclonal antibody used for immunoselection reacts with HLA-A2 but not with HLA-A28, some of the residues that form the BB7.2 epitope should differ in HLA-A28. We suggest that W107 may therefore be a critical residue in forming the BB7.2 antigenic determinant. Although we do not know which residues are altered in mutants 8.18.1 and 8.21.1, we nevertheless can hazard a guess. The 8.18.1 HLA-A2 heavy chain is a single charge more basic than its wild-type counterpart, and the difference peptide has elution properties that indicate an increase in hydrophobicity. These and other pieces of evidence suggest the likelihood of a substitution of a neutral amino acid for an acidic one. Although two acidic residues exist in the wild-type form of the peptide, we suggest that D106, which is within the previously defined variable segment and adjacent to W107, may have changed in 8.18.1. Conversely, because the 8.21.1 HLA-A2 heavy chain is a single
charge more acidic than wild type, and the difference peptide in this case appears less hydrophobic, the substitution here may involve the gain of an acidic residue.

Although residues in this peptide are necessary for the complete expression of the BB7.2 and PA2.1 epitopes, they are certainly not sufficient. Neither antibody binds detectably to wild-type HLA-A2 heavy chains that are not associated with β2m (13; P. Parham, personal communication), presumably because of the loss of native conformation (17). Furthermore, preliminary results indicate that a third BB7.2-immunoselected mutant, 8.6.1, has an alteration in a peptide distant in the linear amino acid sequence from that described here, but nevertheless residing in the same domain of the HLA-A2 heavy chain (S. Taketani and M. S. Krangel, unpublished observations). Thus, other segments of the polypeptide in the second domain may take part directly in the BB7.2 epitope, or possibly stabilize the conformation of residues that are directly involved.

Histocompatibility antigens carry multiple serologically defined alloantigenic determinants. Blocking studies in both the murine and human systems have demonstrated the presence of spatially separate antibody binding sites on the same molecule (18–20). Although BB7.2, PA2.1, and MA2.1 all cross-block (8, 9), their antigenic determinants are nevertheless distinct. The MA2.1 determinant is clearly different from those of PA2.1 and BB7.2 because it is also found on HLA-B17, and because mutants selected with BB7.2 are unaltered in their MA2.1 reactivity. On the other hand, the determinants recognized by BB7.2 and PA2.1 are nearly identical. Although neither antibody reacts with HLA-A28, both react with a presumed HLA-A26 variant cell line, IDF, and immunoselection of T5-1 HLA-A2 mutants with BB7.2 results in an altered PA2.1 determinant. However, the different mutations that characterize 8.18.1 and 8.21.1 can distinguish these antibodies. Thus, comparative binding studies indicate that whereas PA2.1 has a higher affinity for 8.18.1 than for 8.21.1, this situation is reversed for BB7.2 (unpublished observations). The binding of these antibodies to HLA-A2 molecules may involve different contacts, possibly including some different residues, at the same site on the molecule.

Increasing evidence indicates that the determinants defined by serology may not be identical with those recognized by CTL. Thus, H-2Kb mutants with gross alterations in CTL recognition properties have rather limited alterations in serological determinants (21, 22). HLA-A2 structural variants that are not recognized by HLA-A2-restricted, influenza-virus-immune or HLA-A2-specific alloimmune CTL nevertheless have normal reactivities with alloantisera and monoclonal antibodies specific for HLA-A2 (11, 23, 24). Preliminary results indicate HLA-A2-specific alloimmune CTL recognition of immunoselected mutants 8.18.1, 8.21.1, and 8.6.1 may be largely or completely unaffected (C. Ware, personal communication). However, detailed analyses of the reactivities of a battery of HLA-A2-specific CTL clones will be required to more precisely address this question. It is anticipated that extensive primary structural comparisons of allelic products, the generation and analysis of many other variants with altered recognition properties, and ultimately, knowledge of the three-dimensional structure of the molecule should allow a precise understanding of the relationships among these various determinants on the surface of the molecule.

Krangel, M. S., W. E. Biddison, and J. L. Strominger. 1983. Comparative structural analysis of HLA-A2 antigen distinguishable by cytotoxic T lymphocytes. II. Variant DK1: evidence for a discrete CTL recognition region. J. Immunol. In press.
Summary

The HLA-A2-specific mouse monoclonal antibody BB7.2 plus complement has been used to immunoselect variant clones of the lymphoblastoid cell line T5-1 (HLA-A1, -A2, -B8, and -B27). Members of one class of variant clones appear to express cell surface HLA-A2 molecules that display reduced reactivity with the selecting antibody, but normal or near normal reactivities with some other HLA-A2-specific monoclonal antibodies and human alloantisera. The HLA-A2 heavy chains derived from two of these variant clones were characterized by comparative double-label tryptic peptide mapping in conjunction with microsequence analysis. These heavy chains were found to carry distinct mutations in the same peptide in the molecule. We conclude that residues within this short segment of the polypeptide contribute to an HLA-A2-specific serological determinant.

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References

1. Klein, J. 1975. Biology of the Mouse Histocompatibility-2 Complex. Springer-Verlag, New York.
2. Klein, J. 1979. The major histocompatibility complex of the mouse. Science (Wash. D. C.). 203:516.
3. Dorf, M. E., ed. 1981. The Role of the Major Histocompatibility Complex in Immunobiology. Garland Press, New York.
4. Ploegh, H. L., H. T. Orr, and J. L. Strominger. 1981. Major histocompatibility antigens: the human (HLA-A, -B, -C) and murine (H-2K, H-2D) class I molecules. Cell. 24:287.
5. Lopez de Castro, J. A., J. L. Strominger, D. M. Strong, and H. T. Orr. 1982. Structure of crossreactive human histocompatibility antigens HLA-A28 and HLA-A2: implications for a possible mechanism for generation of HLA polymorphism. Proc. Natl. Acad. Sci. U. S. A. 79:3813.
6. Sherman, L. A. 1980. Dissection of the B10.D2 Anti-H-2Kb cytolytic T lymphocyte receptor repertoire. J. Exp. Med. 151:1386.
7. Biddisson, W. E., G. M. Shearer, and S. Shaw. 1981. Influenza virus-specific cytotoxic T cells are restricted by multiple HLA-A3-related self antigens: evidence for recognition of distinct self structures in conjunction with different foreign antigens. J. Immunol. 127:2231.
8. Parham, P., M. J. Androlewicz, F. M. Brodsky, N. J. Holmes, and J. P. Ways. 1983. Monoclonal antibodies: purification, fragmentation and application to structural and functional studies of class I MHC antigens. J. Immunol. Methods. In press.
9. Parham, P., and F. M. Brodsky. 1981. Partial purification and some properties of BB7.2. A cytotoxic monoclonal antibody with specificity for HLA-A2 and a variant of HLA-A28. Human Immunol. 3:277.
10. Krangel, M. S., D. Pious, and J. L. Strominger. 1982. Human histocompatibility antigen mutants immunoselected in vitro. Biochemical analysis of a mutant which synthesizes an altered HLA-A2 heavy chain. J. Biol. Chem. 257:5296.
11. Krangel, M. S., S. Taketani, W. E. Biddison, D. M. Strong, and J. L. Strominger. 1982. Comparative structural analysis of HLA-A2 antigens distinguishable by cytotoxic T lymphocytes. I. Variants M7 and DR1. Biochemistry. In press.
12. Parham, P., and W. F. Bodmer. 1978. Monoclonal antibody to a human histocompatibility alloantigen, HLA-A2. Nature (Lond.). 276:397.
13. Krangel, M. S., H. T. Orr, and J. L. Strominger. 1979. Assembly and maturation of HLA-A and HLA-B antigens in vivo. Cell. 18:979.
14. Orr, H. T., J. A. López de Castro, D. Lancet, and J. L. Strominger. 1979. Complete amino acid sequence of a papain-solubilized human histocompatibility antigen, HLA-B7. 2. Sequence determination and search for homologies. Biochemistry. 18:5711.
15. Robb, R. J., C. Terhorst, and J. L. Strominger. 1978. Sequence of the COOH-terminal hydrophilic region of histocompatibility antigens HLA-A2 and HLA-B7. J. Biol. Chem. 253:3319.
16. McMichael, A. J., P. Parham, N. Rust, and F. Brodsky. 1980. A monoclonal antibody that recognizes an antigenic determinant shared by HLA-A2 and B17. Human Immunol. 1:121.
17. Lancet, D., P. Parham, and J. L. Strominger. 1979. Heavy chain of HLA-A and HLA-B antigens is conformationally labile: a possible role for β2-microglobulin. Proc. Natl. Acad. Sci. U. S. A. 76:3944.
18. Parham, P. 1981. Monoclonal antibodies against two separate alloantigenic sites of HLA-B40. Immunogenetics. 11:131.
19. Ozato, K., P. Henkart, C. Jensen, and D. H. Sachs. 1981. Spatially distinct alldeterminants of the H-2Kk molecule as detected by monoclonal anti-H-2 antibodies. J. Immunol. 126:1780.
20. Lemke, H., and G. J. Hammerling. 1981. Topographic arrangement of H-2 determinants defined by monoclonal hybridoma antibodies. In Monoclonal Antibodies and T Cell Hybridomas. G. J. Hammerling, U. Hammerling, and J. F. Kearney, editors. Elsevier/North Holland Biomedical Press, Amsterdam. 102–109.
21. Klein, J. 1978. H-2 mutations: their genetics and effect on immune functions. Adv. Immunol. 26:55.
22. Nairn, R., K. Yamaga, and S. G. Nathenson. 1980. Biochemistry of the gene products from murine MHC mutants. Annu. Rev. Genet. 14:241.
23. Biddison, W. E., M. S. Krangel, J. L. Strominger, F. E. Ward, G. M. Shearer, and S. Shaw. 1980. Virus-immune cytotoxic T cells recognize structural differences between serologically indistinguishable HLA-A2 molecules. Human Immunol. 3:225.
24. Biddison, W. E., D. D. Kostyu, J. L. Strominger, and M. S. Krangel. 1982. Delineation of immunologically and biochemically distinct HLA-A2 antigens. J. Immunol. 129:730.