The Journal of Immunology

Cutting Edge: HLA-B27 Can Form a Novel β_2-Microglobulin-Free Heavy Chain Homodimer Structure

Rachel L. Allen, Chris A. O'Callaghan, Andrew J. McMichael and Paul Bowness

J Immunol 1999; 162:5045-5048; ;
http://www.jimmunol.org/content/162/9/5045

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Cutting Edge: HLA-B27 Can Form a Novel β_2-Microglobulin-Free Heavy Chain Homodimer Structure[1]

Rachel L. Allen,[2] Chris A. O'Callaghan, Andrew J. McMichael,[3] and Paul Bowness

HLA-B27 has a striking association with inflammatory arthritis. We show that free HLA-B27 heavy chains can form a disulfide-bonded homodimer, dependent on residue Cys^{67} in their extracellular α1 domain. Despite the absence of β_2-microglobulin, HLA-B27 heavy chain homodimers (termed HC-B27) were stabilized by a known peptide epitope. HC-B27 complexes were recognized by the conformation-specific Ab W6/32, but not the ME1 Ab. Surface labeling and immunoprecipitation demonstrated the presence of similar W6/32-reactive free heavy chains at the surface of HLA-B27-transfected T2 cells. HC-B27 homodimer formation might explain the ability of HLA-B27 to induce spondyloarthropathy in β_2-microglobulin-deficient mice. ***The Journal of Immunology,*** **1999, 162: 5045–5048.**

There is a striking association between HLA-B27 and a group of closely related arthritic diseases including ankylosing spondylitis (1). Despite extensive studies, its role in arthritis remains obscure. HLA-B27 is normally found in noncovalent association with β_2-microglobulin (β_2m),[4]-presenting peptides for recognition by CTL. Peptide binds within a groove formed by the α1 and α2 domains of the protein, supported by the α3 domain and β_2m (2).

A direct role for HLA-B27 in arthritis could involve peptide presentation or some unique structural feature (3). The discovery that HLA-B27 transgenic mice develop arthritis in the absence of β_2m and MHC class II implied that "free" HLA-B27 heavy chains might present peptide (4, 5). However, although certain mouse alleles possess this ability (6), no human class I protein has been shown to present Ag in the absence of β_2m.

One unusual feature of HLA-B27 is an unpaired cysteine residue (Cys^{67}) in the extracellular α1 domain. Cys^{67} is not essential for maintaining the structure of the molecule (7), although its location above the B peptide anchor pocket (8) would suggest that it influences peptide binding. It has previously been suggested that in vivo modification of Cys^{67} may lead to an autoimmune response (9).

We show that HLA-B27 can form a Cys^{67}-dependent heavy chain homodimer (HC-B27) in the absence of β_2m. HC-B27 complexes are recognized by the conformation-specific Ab W6/32. Disulfide-bonded W6/32-reactive-free heavy chains are also present on HLA-B27-transfected T2 cells.

Materials and Methods

Protein expression in Escherichia coli

Plasmids pLM1-HLA-B27 and pHN1-β_2m encoding the extracellular domain of HLA-B27 and β_2m were a gift from D. Wiley and D. Garboczi (Harvard University, Cambridge, MA). Plasmid B27B was generated from pLM1-HLA-B27 using primers 5′-TTTGTTGAATTCAGGAGGAAT-3′ and 5′-TTGTGATAAGCTTAACGATGATTCCACACCATTTTCTGTG CATCCAGAATATGATGCAGGGATCCCTCCCAT-3′. Plasmids RAB27S and RAB27SB were generated from pLM1-HLA-B27 and RAB27B using primers 5′-GAGACACAGATCAGCAAGGCCAAGGC-3′ and 5′-GCCT TGGCCTTGCTGATCTGTGTCTC-3′ with the Quikchange system (Stratagene, La Jolla, CA). Proteins were expressed and folded as described (10). Peptide was the HIV-1 gag epitope KWRIIMGLNK. Complexes were purified on a fast protein liquid chromatography system from Pharmacia (Piscataway, NJ); m.w. standards were from Pharmacia.

SDS-PAGE and Western blotting

Gels were blotted onto Hybond-C membrane (Amersham, Little Chalfont, U.K.), stained with HC10 Ab (gift from H. Ploegh, Harvard University), and detected using HRP-conjugated rabbit anti-mouse IgG (Sigma) with ECL reagent (Amersham).

ELISA

ELISA was developed using HRP-conjugated rabbit anti-mouse IgG (Sigma) with 3,3′,5,5′-tetramethyl benzidine substrate (Sigma, St. Louis, MO).

Cell surface labeling and immunoprecipitations

Cells were labeled with sulfo-NHS-biotin (Pierce, Rockford, IL). Cells were lysed in the presence of 5 mM iodoacetamide. Immunoprecipitates resolved by SDS-PAGE were blotted onto Hybond C and detected as above or using ExtrAvidin-HRP (Sigma) with ECL reagent.

Results

HLA-B27 heavy chains form disulfide-bonded homodimers

The extracellular domain of HLA-B27 was used in refolding studies. Unlike other alleles (11), HLA-B27 did not fold with β_2m and known epitopes. Reasoning that this might be due to the unpaired

Human Immunology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, United Kingdom.

Received for publication January 12, 1999. Accepted for publication March 1, 1999.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by the Medical Research Council.

[2] Current address: Immunology Division, Department of Pathology, Tennis Court Road, Cambridge, CB2 1QP, U.K.

[3] Address correspondence and reprint requests to Dr. Andrew McMichael, Human Immunology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford, OX3 9DS, U.K. E-mail address: andrew.mcmichael@ndm.ox.ac.uk

[4] Abbreviations used in this paper: β_2m, β_2-microglobulin: HC-B27, HLA-B27 heavy chain homodimers.

0022-1767/99/$02.00

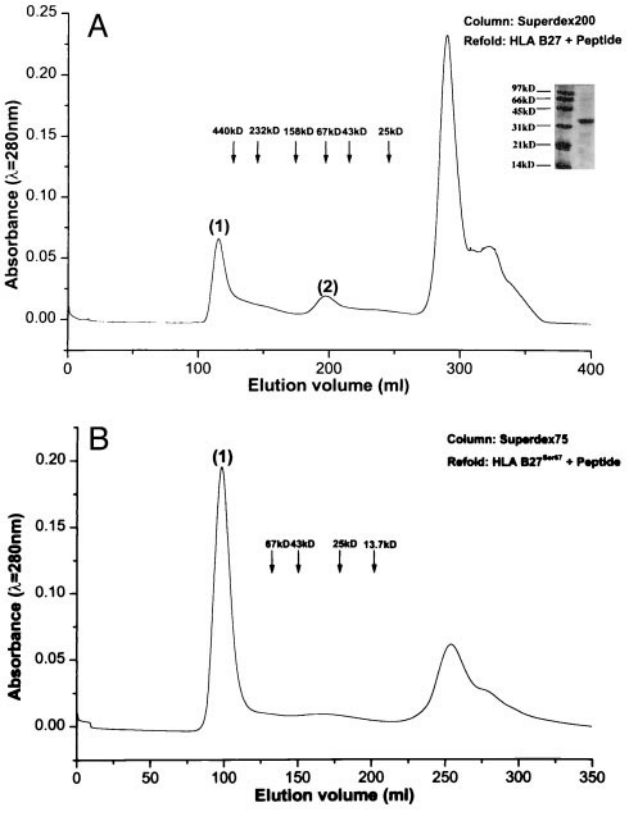

FIGURE 1. Gel filtration of HLA-B27 protein complexes. *A*, HLA-B27 heavy chains refolded without β_2m were analyzed on a Superdex 200 (Pharmacia) column to allow efficient separation of high m.w. species. Aggregates lying outside the limit of resolution elute as a single peak (peak 1). A distinct 66-kDa peak (peak 2) was also identified, and it resolved as a 33-kDa band by reducing SDS-PAGE (*inset*). *B*, A lone aggregate peak was observed in attempts to refold HLA-B27^{Ser67} without β_2m.

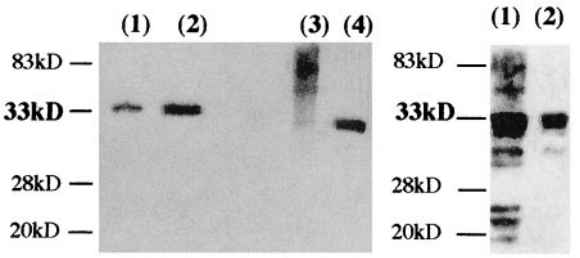

FIGURE 2. HC-B27 dimers are disulfide bonded. Protein complexes were studied by Western blot with the HC10 Ab. *A*, Under reducing conditions, a heavy chain band was observed for both HC-B27 (*lane 1*) and HLA-B27^{Ser67}/β_2m (*lane 2*). SDS-PAGE under nonreducing conditions showed an altered mobility for HC-B27 (*lane 3*), but not for HLA-B27^{Ser67}/β_2m (*lane 4*). *B*, Reducing SDS-PAGE and Western blot of HC-B27 refolded in the absence of peptide detected multiple degradation products (*lane 1*) indicating low stability. A parallel refold differing only by addition of the HIV gag epitope did not show degradation products (*lane 2*).

cysteine, we mutated Cys^{67} to serine (HLA-B27^{Ser67}). HLA-B27^{Ser67} refolded around β_2m and peptide, allowing us to generate tetramer reagents for T cell studies (12). Further analysis of profiles for refolds of HLA-B27^{Cys67} showed an unexpected shoulder of 60–70 kDa. HLA-B27 refolded in the absence of β_2m generated a single 66-kDa peak which resolved as a 33-kDa band by reducing SDS-PAGE (Fig. 1*A*). This peak was absent from refolds using mutated HLA-B27^{Ser67} (Fig. 1*B*). Western blot confirmed that the 66-kDa peak was composed of class I heavy chain (Fig. 2*A*), and its altered mobility under nonreducing conditions indicated disulfide bonding. HC-B27 formed in the absence of added peptide but were unstable (Fig. 2*B*). Addition of the HIV gag epitope KRWIIMGLNK but not the HLA-B27-restricted influenza NP epitope SRYWAIRTR or the HLA-A2-restricted CMV peptide NLVPMVATV to refolds stabilized HC-B27. Acid elution analysis confirmed that the HIV gag peptide was bound by HC-B27 (data not shown).

HC-B27 maintains a stable, W6/32-reactive conformation

Circular dichroism identified secondary structure within the HC-B27 fraction, confirming that complexes were not randomly aggregated (data not shown). We studied the structure of HC-B27 using the heavy chain-specific Ab HC10 and the conformation-specific Abs W6/32 and ME1. HC-B27 showed strong HC10 reactivity (data not shown). W6/32 recognizes residues in the α2 helix of native class I complexes (13), and ME1 recognizes an epitope in the α1 helix of HLA-B27 (3). W6/32 reacted comparably with both HC-B27 and HLA-B27^{Ser67}/β_2m complexes (Fig. 3*A*), demonstrating that HC-B27 can maintain some conformation of its peptide-binding groove. HC-B27 consistently failed to bind ME1. Despite low levels of binding, Ab titration showed that HLA-B27^{Ser67} heterodimers were recognized by ME1 (Fig. 3*B*).

W6/32-reactive, β_2m-free heavy chains can be immunoprecipitated from the surface of HLA-B27-transfected cells

The T2 mutant cell line retains most MHC class I allotypes within the endoplasmic reticulum in a W6/32-unreactive form. T2-HLA-B27 transfectants, however, show unusually high W6/32 reactivity (14, 15), despite low ME1 binding (16). After surface labeling, reducing SDS-PAGE identified β_2m in the W6/32 precipitates of

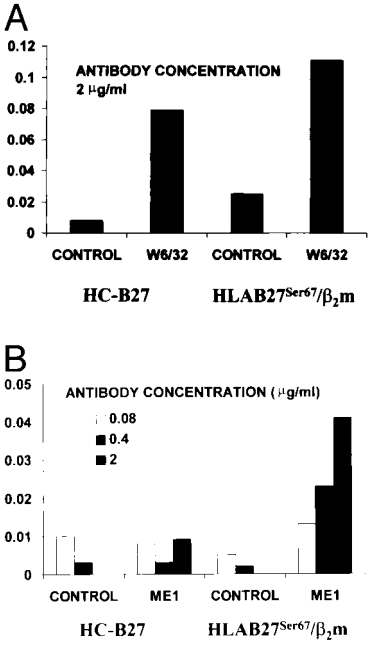

FIGURE 3. ELISA of HLA-B27 protein complexes. Results shown are subtracted from background (BSA). *A*, Both HC-B27 and HLA-B27^{Ser67}/β_2m were bound by the W6/32 Ab. Backgrounds were 0.096 (W6/32) and 0.08 (control). *B*, Only HLA-B27^{Ser67}-β_2m complexes were recognized by the ME1 Ab. Backgrounds were 0.048 (ME1) and 0.06 (control).

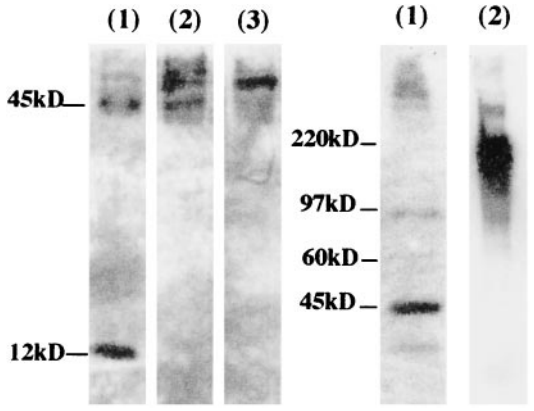

FIGURE 4. Immunoprecipitation of HLA-B27 transfected cell lines. *A*, Lysates of surface-biotinylated cells were precipitated using W6/32. Both 45-kDa heavy chain and 12-kDa β_2m bands were clearly visible for C1R-HLA-B27 cells (*lane 1*). A 45-kDa band was seen in W6/32 precipitates from T2-HLA-B27 transfectants (*lane 2*), but no corresponding β_2m band was observed. Both bands were absent from untransfected T2 cell precipitates (*lane 3*). *B*, W6/32 precipitates of T2-HLA-B27 lysates were analyzed by Western blot with HC10 Ab. The single heavy chain band seen under reducing conditions (*lane 1*) showed an altered mobility under nonreducing conditions (*lane 2*), where a very faint dimer and a predominant tetramer-sized band were observed.

control C1R-HLA-B27 cells, but not in those of T2-HLA-B27 transfectants (Fig. 4*A*). Similarly, a 45-kDa heavy chain band was seen in T2-HLA-B27 precipitates but was absent from untransfected cells. High m.w. bands seen in T2-HLA-B27 precipitates may represent the HLA-B27 coprecipitates previously observed for TAP-deficient cells (17). Western blot of T2-HLA-B27 lysates showed that W6/32 precipitates ran at positions consistent with heavy chain tetramers under nonreducing conditions (Fig. 4*B*). Tetramerization could be explained by disulfide bonding through both Cys^{67} and a second unpaired cysteine in the cytoplasmic tail of the molecule. Class I heavy chains bonded through this residue have been documented previously (18). This could not have taken place in our refolding studies, which used the extracellular domains of HLA-B27.

Discussion

Our findings may be relevant to studies that show that HLA-B27 transgenic mice lacking β_2m develop an arthritis the incidence of which can be reduced by treatment with HC10 Ab (5). HC-B27 formation is dependent on disulfide bonding through Cys^{67}. Such bonding would almost certainly require unwinding of the α1 helix, consistent with loss of the ME1 epitope. However, HC-B27 complexes are recognized by W6/32 and retain an ability to bind peptide, implying that at least part of the groove structure remains intact. Failure of the influenza NP epitope to bind HC-B27 may be explained by alterations to the peptide-binding groove, and it is possible that HC-B27 binds a subset of classically presented epitopes. An interesting possibility is that partial unwinding of the α1 helix could open the groove to accommodate longer peptides. Long peptides were previously shown to bind a subset of HLA-B27 molecules (19). A partially unwound α1 helix might also resemble the α-chain helix of an MHC class II molecule, raising the possibility of CD4 T cell recognition.

Unlike free heavy chains found on the surface of activated T and B cells that lack W6/32 reactivity (20, 21), we have shown that W6/32-reactive heavy chains can be expressed at the surface of T2-HLA-B27. Altered forms of HLA-B27 may be readily detected on this line due to inhibition of normal class I expression. Similar forms may be present on cells such as C1R-HLA-B27 but at too low a level to be easily detected. In that the T2 line is deficient in TAP-transported peptides, it may be that empty HLA-B27 heavy chains can progress to the cell surface. Alternatively, structural alterations could allow peptides from another source to bind. Progression of heavy chains through the endocytic pathway is consistent with their surface expression in β_2m knockout mice (4) and Ag-processing mutants (16).

Aberrant molecules such as HC-B27 could theoretically trigger disease if, e.g., expressed as a neoantigen under certain conditions. The ME1 epitope can be lost during spondyloarthropathy (22, 23) or bacterial infection (24), and it previously has been suggested that Cys^{67} modification could trigger autoimmunity (11, 25). Alternatively, HC-B27 could act through peptide presentation. It will be important to determine whether HC-B27 is recognized by the immune system and, if so, as an Ag-presenting molecule or as a novel autoantigen.

Conclusions

We have shown that HLA-B27 can form a stable homodimer structure (HC-B27). Despite the absence of β_2m, HC-B27 shares some tertiary structure of its peptide-binding groove with conventional complexes and appears to be capable of peptide binding. Similar structures can be expressed on the surface of HLA-B27-transfected cells. HC-B27 is of particular interest due to its potential role in the pathogenesis of HLA-B27-associated disease.

Acknowledgments

We thank D. Wiley, D. Garboczi, E. Collins, and M. Bouvier for plasmids and advice; M. Gross for spectrophotometry; V. Cerundolo, T. Elliott, Y. Jones, and N. Zaccai for discussions; and J. Edwards, G. Gillespie, J. Mongkolsapaya, G. Ogg, L. Schimanski, L. Tan, B. Willcox, J. Wilson, and J. Wyer for protocols and reagents.

References

1. Brewerton, D. A., M. Caffrey, F. D. Hart, D. C. O. James, A. Nichols, and R. D. Sturrock. 1973. Ankylosing spondylitis and HL-A27. *Lancet 1:904.*
2. Madden, D. R. 1995. The three-dimensional structure of peptide-MHC complexes. *Annu. Rev. Immunol. 13:587.*
3. Benjamin R., and P. Parham. 1990. Guilt by association: HLA-B27 and ankylosing spondylitis. *Immunol. Today 11:137.*
4. Khare, S. D., J. Hansen, H. S. Luthra, and C. S. David. 1996. HLA-B27 heavy chains contribute to spontaneous inflammatory disease in B27/human β_2-microglobulin (β_2m) double transgenic mice with disrupted mouse β_2m. *J. Clin. Invest. 98:2746.*
5. Khare, S. D., M. J. Bull, J. Hanson, H. S. Luthra, and C. S. David. 1998. Spontaneous inflammatory Disease in HLA-B27 transgenic mice is independent of MHC class II molecules: a direct role for B27 heavy chains and not B27-derived peptides. *J. Immunol. 60:101.*
6. Bix, M., and D. Raulet. 1992. Functionally conformed free class I heavy chains exist on the surface of β_2 microglobulin negative cells. *J. Exp. Med. 176:829.*
7. el-Zaatari, F. A., K. C. Sams, and J. D. Taurog. 1990. In vitro mutagenesis of HLA-B27: amino acid substitutions at position 67 disrupt anti-B27 monoclonal antibody binding in direct relation to the size of the substitution of the side chain. *J. Immunol. 144:1512.*
8. Madden, D. R., J. C. Gorga, J. L. Strominger, and D. C. Wiley. 1991. The structure of HLA-B27 reveals nonamer self-peptides bound in an extended conformation. *Nature 353:321.*
9. MacLean, L., M. Macey, M. Lowdell, S. Badakere, M. Whelan, D. Perrett, and J. Archer. 1992. Sulphydryl reactivity of the HLA-B27 epitope: accessibility of the free cysteine studied by flow cytometry. *Ann. Rheum. Dis. 51:456.*
10. Garboczi, D. N., D. T. Hung, and D. C. Wiley. 1992. HLA-A2-peptide complexes: refolding and crystallization of molecules expressed in *Escherichia coli* and complexed with single antigenic peptides. *Proc. Natl. Acad. Sci. USA 89: 3429.*
11. McMichael, A. J., and C. A. O'Callaghan.1998. A new look at T cells. *J. Exp. Med. 187:1367.*
12. Wilson, J. D. K., G. S. Ogg, R. L. Allen, P. J. R. Goulder, A. Kelleher, A. K. Sewell, C. A. O'Callaghan, S. L. Rowland-Jones, M. F. C. Callan, and A. J. McMichael. 1998. Oligoclonal expansions of CD8+ T cells in chronic HIV infection are antigen specific. *J. Exp. Med. 188:785.*

13. Maziarz, R. T., J. Fraser, J. L. Strominger, and S. J. Burakoff. 1986. The human HLA-specific monoclonal antibody W6/32 recognises a discontinuous epitope within the α 2 domain of murine H-2Db. *Immunogenetics 24:206.*
14. Colbert, R. A., S. L. Rowland-Jones, A. J. McMichael, and J. A. Frelinger. 1994. Differences in peptide presentation between B27 subtypes: the importance of the P1 side chain in maintaining high affinity peptide binding to B*2703. *Immunity 1:121.*
15. Tan, L., M. H. Andersen, T. Elliott, and J. S. Haurum. 1998. An improved assembly assay for peptide binding to HLA-B*2705 and H-2K^k class I MHC molecules *J. Immunol. Methods 209:25.*
16. Peh, C. A., S. R. Burrows, M. Barnden, R. Khanna, P. Cresswell, D. J. Moss, and J. McCluskey. 1998. HLA-B27-restricted antigen presentation in the absence of tapasin reveals polymorphism in mechanisms of HLA class I peptide loading. *Immunity 8:531.*
17. Raposo, G., H. M. van Santen, R. Leijendekker, H. J. Geuze, and H. L. Ploegh. 1995. Misfolded major histocompatibility complex class I molecules accumulate in an expanded ER-golgi intermediate compartment. *J. Cell Biol. 131:1403.*
18. Capps, G., G. E. Robinson, K. D. Lewis, and M. C. Zuniga. 1993. In vivo dimeric association of class I MHC Heavy chains: possible relationship to class I MHC heavy chain-β_2-microglobulin dissociation. *J. Immunol. 151:159.*
19. Urban, R. G., R. M. Chicz, W. S. Lane, J. L. Strominger, A. Rehm, M. J. H. Kenter, F. G. C. M. Uytdehaag, H. Ploegh, B. Uchanska-Ziegler, and A. Ziegler. 1994. A subset of HLA-B27 molecules contains peptides much longer than nonamers. *Proc. Natl. Acad. Sci. USA 91:1534.*
20. Madrigal, J. A., M. P. Belich, R. J. Benjamin, A.-M. Little, W. H. Hildebrand, D. L. Mann, and P. Parham. 1991. Molecular definition of a polymorphic antigen (LA45) of free HLA-A and -B heavy chains found on the surface of activated T and B cells. *J. Exp. Med. 174:1085.*
21. Demaria, S., R. Scwab, and Y. Bushkin. 1992. The origin and fate of β_2m-free MHC class I molecules induced on activated T cells. *Cell. Immunol. 142:103.*
22. Neumuller, J., M. Fischer, and R. Eberl. 1993. Failure of the serological determination of HLA-B27 due to antigen masking in patients with ankylosing spondylitis. *Rheumatol. Int. 13:163.*
23. Kirveskari, J., H. Kellner, M. Wuorela, H. Soini, B. Frankenberger, M. Leirisalo-Repo, E. H. Weiss, and K. Granfors. 1997. False-negative serological HLA-B27 typing results may be due to altered antigenic epitopes and can be detected by polymerase chain reaction. *Br. J. Rheumatol. 36:185.*
24. Wuorela, M., S. Jalkanen, J. Kirveskari, P. Laitio, and K. Granfors. 1991. *Yersinia enterocolitica* serotype O:3 alters the expression of serologic HLA-B27 epitopes on human monocytes. *Infect. Immun. 65:2060.*
25. Gao, X. M., P. Wordsworth, A. J. McMichael, M. M. Kyaw, M. Seifert, D. Rees, and G. Dougan. 1996. Homocysteine modification of HLA antigens and its immunological consequences. *Eur. J. Immunol. 26:1443.*