# Public Antigenic Determinant on a Family of HLA-B Molecules

## BASIS FOR CROSS-REACTIVITY AND A POSSIBLE LINK WITH DISEASE PREDISPOSITION

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ABSTRACT Serologic cross-reactivity among allelic gene products commonly occurs in the HLA complex, but the molecular basis of these serologic phenomena is incompletely characterized. Because of strong cross-reactivity among antigens comprising the B7 cross-reactive group (i.e., HLA-B7, Bw22, B27, B40, and Bw42) and because of the association of several antigens of this group with spondyloarthropathies, we initiated a study of the chemical basis of cross-reactivity among this group of antigens. Using classic serologic procedures, $^{125}$I-Protein A binding assay, and chemical immunoprecipitation techniques, we have defined a new antigenic determinant, tentatively designated "X", which is present on certain HLA-B molecules. By a series of sequential immunoprecipitation experiments, X was shown to be a "public" antigenic determinant distinct from the "private" determinants B7, Bw22, B27, and B40, but present on the same 44,000-dalton glycoprotein molecules. The implications of this finding regarding disease predisposition and HLA typing as a diagnostic aid are discussed.

## INTRODUCTION

The HLA classic histocompatibility antigens, HLA-A, -B, and -C, were originally discovered because of their ability to elicit antibodies in individuals who had received blood transfusions from a second individual (1). Because the antigens recognized by these antibodies were present in only some individuals of the species, they were alloantigens. Subsequently, they were shown to be the products of a polymorphic genetic system. The predominant role of these antigens in determining the success of tissue transplants stimulated extensive serological definition of this antigen system, as well as genetic definition of the HLA complex determining these antigens. For example, 33 different antigenic determinants have been recognized by the International Nomenclature Committee as being encoded by alleles of the HLA-B locus (2). However, despite this careful definition of HLA antigens, serologic cross-reactivity among various HLA antigens is widespread and well recognized (3). Among the HLA-B antigens, there are multiple distinct groups of cross-reacting antigens. However, few, if any, studies have attempted to elucidate the chemical basis of this serologic cross-reactivity.

Over the past several years, intense interest in the HLA classic histocompatibility antigens has been rekindled by the findings that these antigens act as target molecules of cell-mediated cytolysis (4), that these antigens play a crucial role in the phenomenon of histocompatibility restriction of cell-mediated cytolysis in viral and minor histocompatibility systems (5, 6), and particularly by the observation that certain of these antigens are associated with certain disease states. The most striking association is that of ankylosing spondylitis with HLA-B27 (7); 90% of Caucasian patients with this disease possess HLA-B27. Similar findings have been made in Reiter's syndrome in which ≅70% of Caucasian patients have HLA-B27, and idiopathic sacroiliitis in which 50% of patients are HLA-B27$^+$ (8). Recently, two studies have examined patients with

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these three disease states, who lacked the HLA-B27 antigen (9, 10). In the first study, 70% of HLA-B27$^-$ patients possessed one of three antigens: B7, Bw22, or Bw42 (9). In the second study, 56% of black patients with ankylosing spondylitis lacking HLA-B27 were found to have B7 (10). The surprisingly high frequency of these three antigens in B27$^-$ patients with spondyloarthropathy is especially intriguing because B7, Bw22, B27, and Bw42 all belong to a serologically cross-reactive group of HLA-B antigens which also includes HLA-B40 (3).

Because this cross-reactive group of HLA-B antigens was apparently associated with at least a subgroup of spondyloarthropathies, and because the basis of serologic cross-reactivity in the HLA system remained largely undefined, we undertook a study to establish the chemical basis of cross-reactivity for this group of antigens. Our studies demonstrate that HLA-B7, Bw22, B27, and B40 molecules all share a common, but distinct antigenic determinant.

## METHODS

*Cells.* Peripheral blood cells were obtained from healthy volunteers who had previously been typed for HLA-A and -B determinants. Table I lists the HLA types of individuals whose cells were used as a source of various HLA-B antigens. Unfortunately, no individual with HLA-Bw42 was available for our study. In collecting cells, ≅50 ml of blood was immediately heparinized with 0.1–0.2 ml ammonium heparin (1,000 U/ml) (Sherwood Medical Industries, Inc., St. Louis, Mo.). 25 ml of heparinized blood was mixed with 10 ml 6% dextran in normal saline (McGraw Laboratories, Glendale, Calif.), and allowed to stand for 60 min at 37°C. At the end of this time, the top 15–20 ml was collected, washed, and used as the source of leukocytes in subsequent steps (11).

TABLE I

*HLA Types of Individuals Used as a Source of Various HLA-B Antigens*

| HLA-B antigen studied | HLA type of individuals |
|---|---|
| B7 | A2, A3, B7, B35 |
| | A2, --, B7, B35 |
| Bw22 | A1, --, B17, Bw22 |
| | A11, A31, Bw22, B35 |
| B27 | A2, A3, B12, B27 |
| | A23, A26, B21, B27 |
| B40 | A2, --, B12, B40 |
| | A28, A29, B17, B40 |
| B7, B27 | A3, A31, B7, B27 |
| | A1, A3, B7, B27 |
| B7, B40 | A3, A28, B7, B40 |
| Controls | A24, A29, B38, B-- |
| | A2, A24, B15, B35 |
| | A1, A--, B8, B35 |
| | A24, A29, B12, B38 |
| | A3, A--, B12, B14 |

TABLE II

*Titers of Serum BB, Anti-X, and Anti-B7 on Cells Bearing HLA-B Antigens of the B7 Cross-Reacting Group*

| | Cells tested | | | |
|---|---|---|---|---|
| | B7 | Bw22 | B27 | B40 |
| Serum BB | 1:32 | NR* | 1:8 | NR |
| Anti-X | 1:8 | NR | 1:8 | NR |
| Anti-B7 | 1:32 | NR | NR | NR |

* NR, no reaction with undiluted serum or antibody.

*Alloantiserum.* Serum BB was obtained from a multiparous female (HLA-A1, A28, B13, B14) whose husband was typed as HLA-A3, A24, B7, B27. This serum when characterized in a microcytotoxicity assay (12) reacted with HLA-B7 cells at a titer of 1:32 and with HLA-B27 cells at a titer of 1:8 (Table II). To separate different antibody populations within the serum, the serum was twice absorbed with B7$^-$, B27$^+$ platelets (13) (actual HLA typing of platelets used was HLA-A3, A9, B14, B27, and HLA-A3, –, B12, B27) as follows. The platelets from one platelet transfer pack (≅7–8 × $10^{10}$ platelets; 2 ml packed volume) were used for each absorption. The platelets were thoroughly washed with acid citrate dextrose solution–Formula A (Fenwal Inc., Walter Kidde & Co. Inc., Ashland, Mass.) diluted 1:5 in normal saline. The packed platelets were then resuspended in 60 ml of serum BB, and incubated at 37°C for 2 h. The platelets were removed by centrifugation at 12,000 *g* for 15 min. The once absorbed serum was then mixed with a second 2 ml of fresh packed platelets, and the absorption step was repeated. The twice absorbed serum when retested in a microcytotoxicity assay still reacted with HLA-B7 cells at a titer of 1:32, but had completely lost its reactivity on HLA-B27 cells (Table II); this twice absorbed serum was therefore used as a functionally monospecific anti-HLA-B7.

The antibody which had reacted with the platelets used in the first absorption step was eluted by resuspending the platelets in 2 ml of physiologic saline, the pH of which had been made 3.0 with HCl (13). The platelets were incubated for 3 min with shaking, and then removed by centrifugation at 12,000 *g* for 10 min. The pH of the eluate was adjusted back to 7.0 with NaOH. When retested in a microcytotoxicity assay, the eluted antibody reacted with both HLA-B7 and HLA-B27 cells at a titer of 1:8 (Table II). This antibody was tentatively designated anti-X.[1] Evidence of its functional monospecificity in this system is given in Results.

The anti-HLA-B27 was the kind gift of Dr. Andrew Goldstein, University of Oregon Health Sciences Center, Portland, Or. This serum, Yarbrough anti-B27, has been thoroughly characterized in the first HLA Workshop of the Americas. It is a functionally monospecific reagent, and gives no reaction on B7, Bw22, or B40 cells.

Anti-HLA-Bw22 was the kind gift of Dr. John Lee, American Red Cross, St. Louis, Mo. This serum has no reactivity against B7, B27, or B40 cells. Anti-HLA-B40 was generously supplied

[1] The designations of the public determinant as "X" and the antibody recognizing this determinant as "anti-X" are used tentatively pending the assignment of a more formal designation by the International Nomenclature Committee.

by Dr. Don Cross, Midwest Organ Bank, Kansas City, Mo. It has no reaction with B7, Bw22, or B27 cells.

*Absorption of anti-X with leukocytes.* 50 μl of anti-X was incubated with $2 \times 10^7$ leukocytes for 60 min at 37°C, and the cells removed by centrifugation at 1,100 *g* for 10 min. This procedure was repeated once, so that in all cases where absorption was necessary, the anti-X was absorbed twice.

*Preparation of an immunoglobulin (Ig)G fraction from normal human serum.* In cases where the human $\beta_2$-microglobulin present in normal serum might interfere with the reaction of rabbit anti-$\beta_2$-microglobulin in the test step of sequential immunoprecipitation experiments, a $\beta_2$-microglobulin-free IgG fraction of the serum was used. This fraction was prepared as follows (14). 1 vol of serum was incubated with 10 vol of a 10% suspension of Protein-A bearing *Staphylococcus aureus* Cowan I strain (SaCI)[2] for 30 min at 4°C. After thorough washing, the IgG was dissociated by the addition of 1 vol of 3.5 M $MgCl_2$ for 15 min at room temperature (15), and the SaCI pellet was removed by centrifugation. The supernate containing the purified IgG was dialyzed against tris-buffered saline, pH 7.4. This dialysate was used as the IgG fraction in subsequent experiments.

*Xenoantiserum.* Rabbit anti-human $\beta_2$-microglobulin was purchased from Accurate Chemical & Scientific Corp., Hicksville, N. Y.

*Human $\beta_2$-microglobulin.* Human $\beta_2$-microglobulin was the generous gift of Dr. Nobuyuki Tanigaki, Roswell Park Memorial Institute, Buffalo, N. Y.

*Radiolabeling of cells and preparation of solubilized antigens.* The procedure used has been previously described (13, 16, 17). In brief, peripheral blood leukocytes were resuspended at $2-5 \times 10^7$ cells/ml in modified Eagle's medium without serum in which the sole source of leucine was [$^3$H]-leucine (80–110 Ci/mM, New England Nuclear, Boston, Mass.) at a concentration of 200 μCi/ml, and incubated for 4–6 h at 37°C in a humidified 5% $CO_2$ atmosphere. The cells were harvested, washed, and then solubilized in tris-buffered saline containing 0.5% of the nonionic detergent Nonidet P-40 (Particle Data Inc., Elmhurst, Ill.). Insoluble debris was removed by ultracentrifugation at 100,000 *g* for 60 min. The glycoprotein molecules containing glucose or mannose were then purified 10- to 20-fold by passage over an affinity column of *Lens culinaris* lectin which had been covalently attached to Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.), and were eluted from the column with 0.1 M α-methyl mannoside (18). This purified glycoprotein fraction was incubated with 300–500 μl packed SaCI for 30 min at 4°C to remove any endogenously radiolabeled IgG. The SaCI were removed by centrifugation, and the supernate was used as the source of solubilized antigen in subsequent steps (17).

*Immunoprecipitation and analysis of solubilized HLA molecules.* An aliquot of glycoprotein fraction containing 100,000–200,000 cpm was incubated with 100 μl of alloantiserum (or eluted alloantibody) for 2 h at 4°C. 1 ml of a 10% suspension of SaCI was then added to pellet any antigen-antibody complexes formed and the incubation was continued an additional 30 min (17). The pellets were centrifuged at 1,100 *g* for 10 min, and washed three times in tris-buffered saline containing 0.25% NP-40. Radiolabeled antigen was then dissociated from antibody and the staphylococci by boiling in 100 μl 0.0625 M Tris, pH 6.8, containing 2% sodium dodecyl sulfate (SDS) and 2% 2-mercaptoethanol for 1.5 min, and the staphylococci removed by centrifugation. 100 μl of 2% SDS, 2% 2-mercaptoethanol, 20% glycerol, and 0.004% phenol red were added, and the sample analyzed in a modification (15) of the discontinuous SDS-polyacrylamide gel electrophoresis (SDS-PAGE) system originally described by Laemmli (19) and Maizel (20). Both 10 and 15% polyacrylamide gels were used. The gels were cut into 2-mm slices, each slice incubated overnight at 37°C in 1.5 ml of toluene containing 7% Protosol (New England Nuclear) and 4 g/liter Omnifluor (New England Nuclear) and the radioactivity of each slice determined in a Beckman LS 8000 scintillation counter (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Molecular weights were estimated from the position of marker proteins electrophoresed at the same time (20).

*Preparation of $^{125}$I-labeled Protein A.* Protein A derived from SaCI (Pharmacia Fine Chemicals) was iodinated using $^{125}$I-Bolton-Hunter reagent (21) (New England Nuclear).

*$^{125}$I-Protein A binding assay (22).* Anti-X and normal human IgG were ultracentrifuged immediately before use to remove material >10S (23). 25 μl of anti-X (an amount known to be in excess) or normal human Ig was incubated with 500,000 leukocytes for 30 min at 4°C. The cells were washed three times with Hanks' balanced salt solution containing 2% ovalbumin, 1,000,000 cpm of $^{125}$I-Protein A was added, and the incubation continued for 30 min at 4°C. The cell pellets were then washed three times, and the radioactivity bound to each cell pellet was determined. Each test was done in triplicate, and average values and standard deviations are reported.

## RESULTS

*Serum BB contains two functionally defined antibody populations.* The reactivities of serum BB against B7 cells and B27 cells at different initial titers suggested that this serum contained two distinct antibody populations. This contention was confirmed by twice absorbing serum BB with HLA-B7$^-$, B27$^+$ platelets, and demonstrating that the absorbed serum would react only with B7$^+$ cells (Table II). In contrast, the antibody(ies) eluted from the platelets reacted with both B7$^+$ and B27$^+$ cells, suggesting that a single antibody population designated anti-X, might be reacting with both HLA-B7 and HLA-B27 cells. However, because the husband of BB was typed as both B7 and B27, it was equally possible that the eluted antibodies could contain anti-HLA-B27 in addition to anti-X.

To ascertain which of these possibilities was correct, a series of absorptions was carried out. Anti-X was twice absorbed with HLA-B7 cells, HLA-B27 cells, or control cells, and then tested for residual activity against B7$^+$ and B27$^+$ cells. In both cases, absorption of anti-X with either B7 or B27 cells completely removed activity for both cell types (Table III). This result indicated that the platelet eluate contained only a functionally monospecific antibody, anti-X, reactive with both B7 and B27 cells, and contained no distinct antibody reactive only with HLA-B27 cells.

*Anti-X reacts with cells bearing HLA-B7, HLA-Bw22, HLA-B27, and HLA-B40.* It was clear from the initial testing of anti-X that it reacted with HLA-B7 and B27 cells as measured by direct cytotoxicity. How-

[2] *Abbreviations used in this paper*: $\beta_2\mu$, $\beta_2$-microglobulin; NHS, normal human serum; PAGE, polyacrylamide gel electrophoresis; SaCI, *Staphylococcus aureus* Cowan I strain; SDS, sodium dodecyl sulfate.

TABLE III
*Absorption of Anti-X by B7 and B27 Cells*

| Undiluted anti-X absorbed twice with cells bearing: | Cells staining with eosin | |
|---|---|---|
| | B7 | B27 |
| | % | |
| B38, B- | 80 | 80 |
| B7 | <10 | <10 |
| B27 | <10 | <10 |

ever, when other members of the B7 cross-reacting group were tested in the same assay, no reactivity was seen (Table II). Because the cytotoxicity-negative, absorption-positive phenomenon is well known in HLA serology (24), we tested whether the cytotoxic activity of anti-X for B7 cells could be removed by absorption with either Bw22- or B40-bearing cells (Table IV). In both cases, the cells completely removed the cytotoxic activity for B7 cells, whereas absorption with control cells only diminished the titer by one dilution.

*$^{125}$I-Protein A binding.* To confirm the results suggested by the direct cytotoxicity and absorption results, and to try to quantitate the qualitative difference indicated by those results, we performed an $^{125}$I-Protein A binding assay. Table V shows one representative experiment of four which were performed. When comparing the binding of one antibody on a number of different cells, the amount of $^{125}$I bound is a reflection of the number of available antigenic sites (22). It appeared that there was a hierarchy of this availability as follows: B27 > B7 > B40 > Bw22. In addition, it appeared that the binding by cells bearing both B7 and B27 was somewhat greater than the sum of the binding by B7 and B27 cells, respectively, and that the binding by cells bearing both B7 and B40 was approximately the sum of the binding by B7 and B40 cells, respectively. We therefore concluded that all cells bearing at least one of the four B7-cross-reactive group antigens which we tested, reacted with anti-X, and that the degree of reactivity of the cell with anti-X was predicated on which HLA-B antigen the cell bore.

TABLE IV
*Absorption of Anti-X by Bw22 and B40 Cells*

| Anti-X absorbed with cells bearing: | B7 cells staining with eosin serum dilution | | | |
|---|---|---|---|---|
| | Neat | 1:2 | 1:4 | 1:8 |
| | % | | | |
| No absorption | >90 | >90 | 60–80 | 40–50 |
| B38/B- | >90 | 70–90 | 40–50 | <10 |
| Bw22 | <10 | <10 | <10 | <10 |
| B40 | <10 | <10 | <10 | <10 |

TABLE V
*Reaction of Anti-X with Various Cells as Assessed by $^{125}$I-Protein A Binding*

| Cells | Anti-X | Normal human IgG | Net‡ |
|---|---|---|---|
| | | *cpm** | |
| B7/B27 | 67,110±5,269 | 7,624±1,230 | 59,486 |
| B7/B40 | 32,077±1,698 | 6,157±586 | 25,920 |
| B27/B21 | 25,726±3,897 | 6,175±994 | 19,551 |
| B7/B35 | 21,912±1,157 | 4,243±886 | 17,669 |
| B40/B12 | 16,988±2,063 | 6,519±1,275 | 10,469 |
| B22/B17 | 11,177±2,055 | 6,943±1,634 | 4,234 |
| B12/B14 (Control) | 9,427±3,311 | 6,471±1,279 | 2,956 |
| B15/B35 (Control) | 10,017±508 | 7,574±1,356 | 2,443 |

* Mean of triplicates ±SD.
‡ Net counts per minute equals mean counts per minute obtained with anti-X minus mean counts per minute obtained with normal human IgG on each cell type.

*Anti-X reacts with an HLA-like molecule.* The reactivity of anti-X with cells bearing a B7-cross-reactive group antigen suggested that anti-X might be cross-reactive at the molecular level. We therefore initiated a series of chemical studies. Fig. 1 shows a chemical confirmation of the efficacy of the platelet absorption separating anti-B7 from anti-X reactivities. Normal human serum (NHS), serum BB, the eluted anti-X, and the twice absorbed anti-B7 were reacted with an antigen

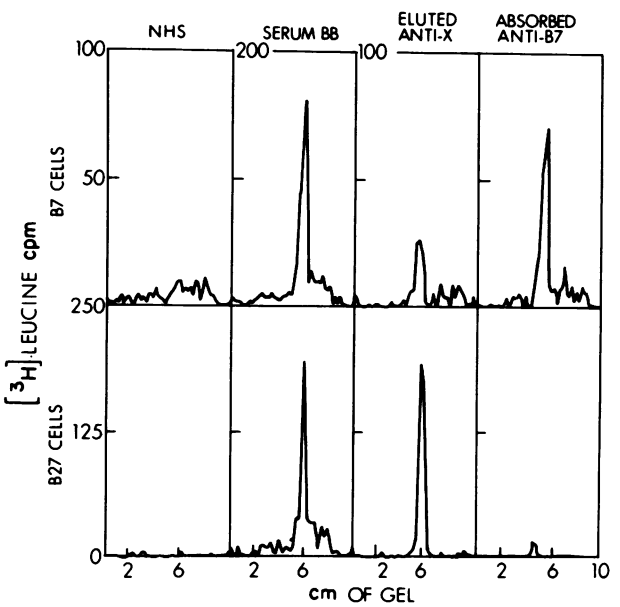


FIGURE 1 Discontinuous SDS-10% PAGE patterns of the molecules solubilized from [$^3$H]leucine-labeled HLA-B7$^+$ cells (top) or HLA-B27$^+$ cells (bottom), and reactive with normal human serum, serum BB, the eluted anti-X, and the twice absorbed anti-B7. The molecules detected all migrate with an apparent mol wt of 44,000 daltons. $\beta_2\mu$ cannot be identified on 10% polyacrylamide gels.

preparation derived from B7$^+$ cells (top row) or with a preparation derived from B27$^+$ cells (bottom row). No peaks are seen with the NHS controls. On the B7 antigen preparation, serum BB, anti-X, and anti-B7 all react with a molecule(s) which migrate(s) with an apparent mol wt of 44,000 ($\beta$2-microglobulin cannot be resolved on a 10% polyacrylamide gel in this system—see below). In contrast, on the B27 antigen preparation, only serum BB and anti-X react with such a molecule, showing the completeness of the initial absorption in yielding a functionally monospecific anti-B7.

The molecular weight estimate of 44,000 was in agreement with the known molecular weight of the HLA-A, -B, and -C heavy chains. To determine if a $\beta_2$-microglobulin ($\beta_2\mu$)-like component was also present, we reacted anti-X with an antigen preparation derived from B7, B27 cells, and analyzed the results on a 15% polyacrylamide gel. Fig. 2 shows an electropherogram containing two peaks. The first component (2 cm) migrates with an apparent mol wt of 44,000, whereas the second component (5 cm) migrates with an estimated mol wt of 12,000, similar to that of $\beta_2\mu$.

*Anti-X and anti-$\beta_2\mu$ recognize the same molecular complex.* To prove that the small component was in fact $\beta_2\mu$, and to prove that anti-X and anti-$\beta_2\mu$ were reacting with the same molecular complex, a sequential immunoprecipitation experiment (25) was performed. This technique tests whether two antigenic determinants are on the same or different molecules, and is shown in Fig. 3.

An antigen preparation prepared from HLA-B7, B27 cells was divided into three aliquots. The first aliquot was pretreated with normal human IgG as a control, the second aliquot was pretreated with anti-$\beta_2\mu$ to remove all molecules having $\beta_2\mu$, and the third aliquot

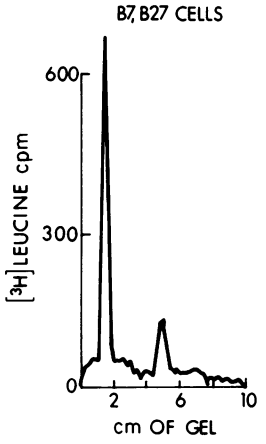


FIGURE 2 Discontinuous SDS-15% PAGE pattern of the molecules solubilized from a [$^3$H]leucine-labeled HLA-B7, B27 cells, and reactive with anti-X. The larger component (2 cm) has an estimated mol wt of 44,000 whereas the smaller component (5 cm) has an estimated mol wt of 12,000.

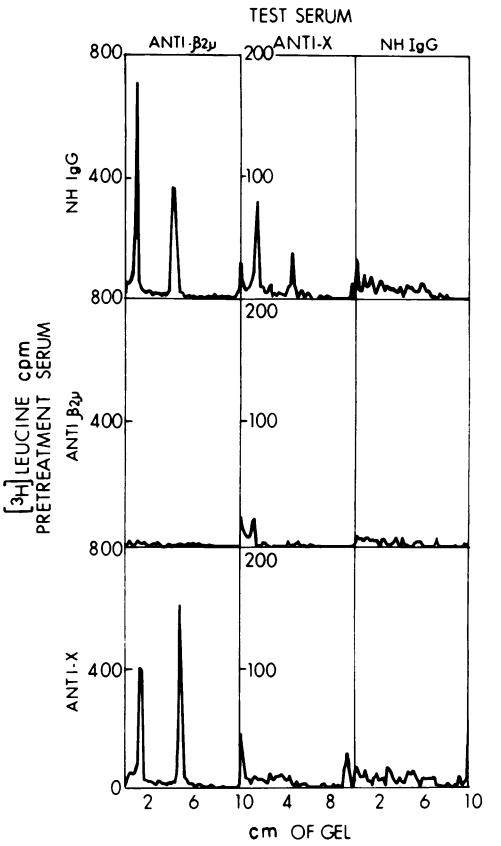


FIGURE 3 Discontinuous SDS-15% PAGE patterns of the molecules solubilized from [$^3$H]leucine-labeled HLA-B7, B27 cells, and reacted in a sequential immunoprecipitation experiment with normal human IgG, anti-X, or anti-$\beta_2\mu$. After pretreatment with normal human IgG (top row), anti-$\beta_2\mu$, and anti-X both react with molecules consisting of a 44,000-dalton chain and a 12,000-dalton chain. Pretreatment with anti-X (bottom row) removes all subsequent activity for itself, but leaves molecules reactive with anti-$\beta_2\mu$. Pretreatment with anti-$\beta_2\mu$ (middle row) removes all molecules reactive with itself and anti-X.

was pretreated with anti-X to remove all molecules bearing determinant X. Each of these pretreated samples was in turn divided into three aliquots, and tested with anti-$\beta_2\mu$ for the presence of $\beta_2\mu$ molecules, with anti-X for the presence of X-bearing molecules, or with normal human IgG as a control. After pretreatment with normal human IgG (top row), both anti-$\beta_2\mu$ and anti-X react with molecules consisting of a 44,000- and 12,000-dalton chain. Pretreatment with anti-X (bottom row) removes all molecules reactive with itself, but does leave molecules reactive with anti-$\beta_2\mu$. This, of course is the expected result, because anti-$\beta_2\mu$ reacts with HLA-A and -C molecules as well as HLA-B molecules. In contrast, pretreatment with anti-$\beta_2\mu$ (middle row) completely clears all molecules reactive with itself or with anti-X. We therefore concluded that anti-X was reacting

with an HLA-like molecule consisting of a heavy chain and $\beta_2\mu$.

*Anti-X reacts with a determinant on the heavy chain.* Although no polymorphism has been described for human $\beta_2\mu$, it was still possible, though unlikely, that anti-X was reacting with a determinant on the $\beta_2\mu$. To determine if anti-X was reacting with a determinant on the heavy chain or on the $\beta_2\mu$, anti-$\beta_2\mu$, and anti-X were preincubated either without or with a vast excess (12 $\mu$g) of free human $\beta_2\mu$, before addition to a B7, B27 antigen preparation (Fig. 4). In the absence of $\beta_2\mu$ (left panels), both anti-$\beta_2\mu$ and anti-X react with the HLA-like complex. Preincubation with $\beta_2\mu$ (right panels) completely blocks the reactivity of anti-$\beta_2\mu$, but leaves the activity of anti-X undiminished. This result indicated that X was a determinant on the heavy chain of these molecules.

*Anti-X reacts with molecules bearing HLA-B7, HLA-B27, HLA-Bw22, and HLA-B40.* The demonstration that anti-X reacted with an HLA-like molecule containing $\beta_2\mu$ suggested that it might be reacting with HLA molecules themselves. However, in the mouse model, several other molecules (e.g., TL and Qa-3) besides H-2D and H-2K have been shown to have a structure similar to that of the major histocompatibility complex antigens (26, 27). Therefore, to determine whether X was a determinant on the HLA-B molecules belonging to the B7 cross-reactive group, we performed a series of sequential immunoprecipitations. Fig. 5 shows the

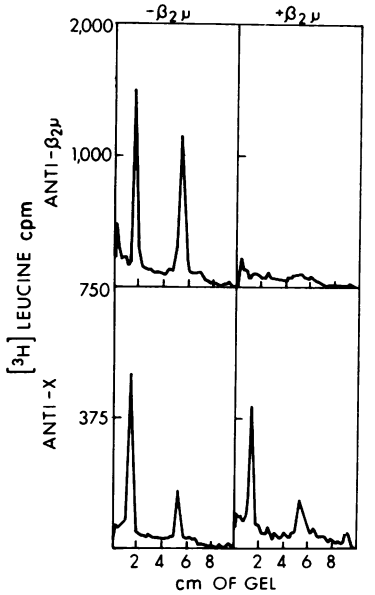


FIGURE 4 Discontinuous SDS-15% PAGE patterns of the molecules solubilized from [$^3$H]leucine-labeled HLA-B7, B27 cells, and reactive with anti-$\beta_2\mu$ (top) or anti-X (bottom) in the absence (left) or presence (right) of free human $\beta_2\mu$. Extrinsic $\beta_2\mu$ blocks the reactivity of anti-$\beta_2\mu$ but doesn't affect the reactivity of anti-X.

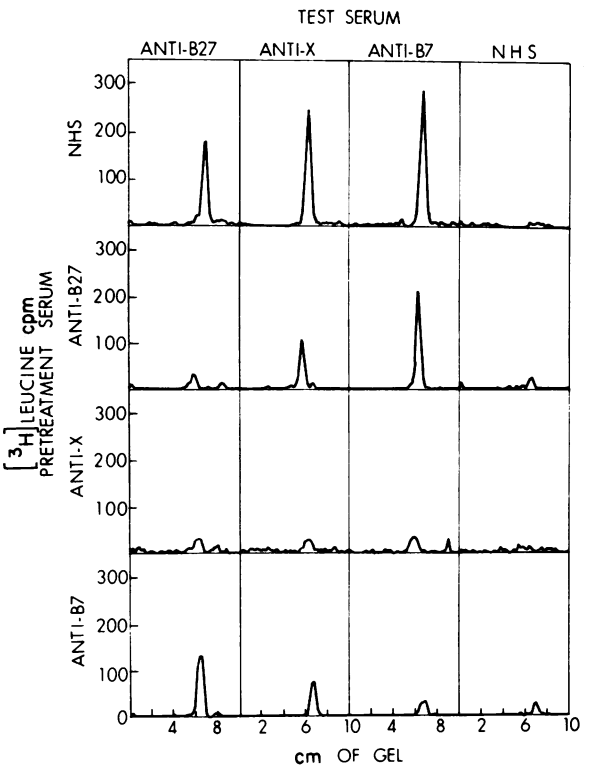


FIGURE 5 Discontinuous SDS-10% PAGE patterns of the molecules solubilized from [$^3$H]leucine-labeled HLA-B7, B27 cells, and reacted in a sequential immunoprecipitation experiment with NHS, anti-B27, anti-X, and anti-B7. After pretreatment with NHS (top row) molecules bearing B27, X, and B7 can all be readily detected by the appropriate antibody. Anti-B27 (second row) clears virtually all molecules reactive with itself, but leaves molecules reactive with anti-X and anti-B7. Pretreatment with anti-B7 (bottom row) depletes essentially all molecules reactive with itself, but leaves molecules reactive with anti-B27 and anti-X. Pretreatment with anti-X (third row) removes virtually all molecules reactive with all three antibodies.

SDS-PAGE patterns of such an experiment when anti-X, anti-B27, anti-B7, and NHS were reacted with an antigen preparation derived from B7, B27 cells.

After pretreatment with normal human serum (top row), anti-B27, anti-X, and anti-B7 all react with molecules of 44,000 daltons. (Again, the $\beta_2\mu$ cannot be seen on 10% polyacrylamide gels). Pretreatment with anti-B27 (second row) removes virtually all molecules reactive with itself, but leaves behind molecules reactive with anti-X and anti-B7. Conversely, pretreatment with anti-B7 (fourth row) depletes virtually all molecules reactive with itself, but leaves molecules reactive with anti-B27 and anti-X. In contrast, pretreatment with anti-X (third row) removes virtually all molecules reactive with all three antibodies. We therefore concluded that anti-X was reacting with a determinant designated "X" which was on the same glycoprotein chain as HLA-

B7, and on the same glycoprotein chain as HLA-B27, but was distinct from both the HLA-B7 and HLA-B27 determinants. These results were confirmed by sequential immunoprecipitation experiments on antigen preparations containing B7 (without B27) or B27 (without B7) (data not shown).

To determine whether X might also be on other HLA molecules other than HLA-B molecules, a second sequential immunoprecipitation was conducted with the antigen preparation derived from B7, B27 cells (Fig. 6). After pretreatment with normal human IgG (top row), anti-B7, anti-B27, and anti-X again react with molecules of 44,000 daltons. Pretreatment with a combination of anti-B7 and anti-B27 completely removed all molecules reactive with any of the antibodies, including anti-X. Thus, on this cell, no molecular species other than HLA-B7 and HLA-B27 was reactive with anti-X. This result indicated that X was a determinant which appeared to be confined to HLA-B molecules, and was not present on HLA-A or HLA-C molecules.

Because of the paucity of functionally monospecific anti-Bw22 and anti-B40 antisera, only one-way sequential immunoprecipitation studies could be performed on these molecules. Figs. 7 and 8 show the SDS-PAGE patterns from these experiments. In Fig. 7, pretreatment with normal human serum (top row) leaves molecules reactive with both anti-Bw22 and anti-X, whereas treatment with anti-X (bottom row) removes virtually

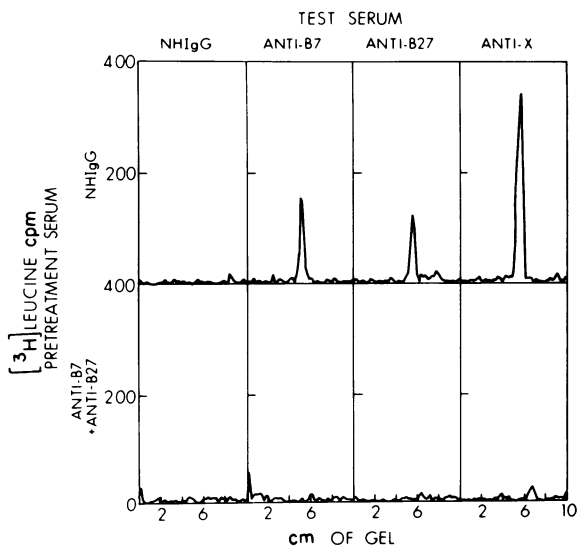


FIGURE 6 Discontinuous SDS-10% PAGE patterns of molecules solubilized from [$^3$H]leucine-labeled HLA-B7, B27 cells and reacted in a sequential immunoprecipitation experiment with normal human IgG, anti-B7, anti-B27, and anti-X. Control pretreatment (top row) leaves molecules reactive with all three antibodies. Pretreatment with a combination of anti-B7 and anti-B27 (bottom row) removes all molecules reactive with all three antibodies.

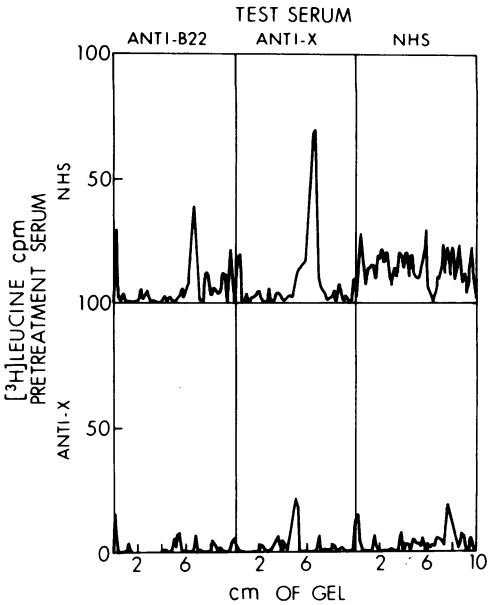


FIGURE 7 Discontinuous SDS-10% PAGE patterns of molecules solubilized from [$^3$H]leucine-labeled HLA-Bw22 cells, and reacted in a sequential immunoprecipitation experiment with NHS, anti-Bw22, and anti-X. Pretreatment with NHS (top row) leaves molecules reactive with both anti-Bw22 and anti-X. Pretreatment with anti-X (bottom row) removes virtually all molecules reactive with both antibodies.

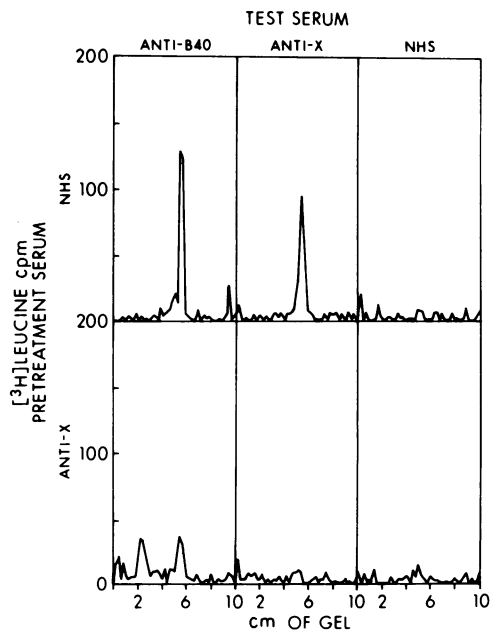


FIGURE 8 Discontinuous SDS-10% PAGE patterns of molecules solubilized from [$^3$H]leucine-labeled HLA-B40 cells and reacted in a sequential immunoprecipitation experiment with NHS anti-B40, or anti-X. Control pretreatment (top row) leaves molecules reactive with both anti-B40 and anti-X. Pretreatment with anti-X (bottom row) depletes essentially all molecules reactive with both antisera.

all molecules reactive with both antibodies. Similarly, in Fig. 8, after control pretreatment (top row), molecules reactive with anti-B40 and anti-X are readily detected; pretreatment with anti-X (bottom row) depletes essentially all molecules reactive with both antibodies. We therefore concluded that X was a distinct determinant on the glycoprotein component bearing HLA-Bw22, and on the glycoprotein component bearing HLA-B40.

*Anti-X does not react with all HLA-B molecules.* The demonstration that X was a determinant on all tested HLA-B molecules belonging to the B7 cross-reacting group did not indicate whether or not X might also be a determinant on other HLA-B molecules. We therefore prepared labeled antigen preparations from cells of several individuals who did not possess one of the B7-cross-reactive group antigens. Although, the HLA-B molecules in question could be readily detected by appropriate anti-HLA alloantisera and/or rabbit anti-$\beta_2\mu$, thus establishing a positive control, anti-X did not react with B8-, B12-, B15-, B35-, or B38-bearing molecules (data not shown). These results strongly suggested that X was a determinant confined to the B7 cross-reacting group.

## DISCUSSION

The studies presented in this communication demonstrate that HLA-B7, Bw22, B27, and B40 molecules all share a common, but distinct antigenic determinant, which we have tentatively designated X (Fig. 9). The demonstration of this common determinant firmly establishes a chemical basis for serologic cross-reactivity for this subgroup of HLA-B molecules, and suggests that similar common determinants will be found which will explain the serologic cross-reactivity of other groups of HLA molecules.

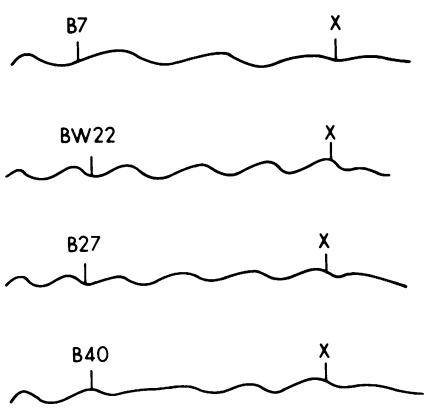


FIGURE 9 Schematic representation of our concept of the public antigenic determinant, X, on the same 44,000-dalton glycoprotein chains as HLA-B7, Bw22, B27, and B40.

The concept of common antigenic determinants on a subgroup of HLA molecules has been previously explored by Legrand and Dausset (28, 29). These workers, using classic serological and capping techniques, postulated one common determinant on HLA-B5, B15, B17, and B21 molecules (28), and a second common determinant on HLA-A24, B5, B17, B21, and B27 cells (29), and suggested that chemical studies be done to confirm this postulate.

Although this is the first chemical demonstration of a common determinant confined to the molecules of a cross-reacting group, common determinants on a broader range of HLA-B molecules have been previously seen. Thus, HLA-Bw4 and Bw6, which originally were defined as the products of a diallelic system (30), on further study, using serologic techniques, appeared to be alternative antigenic determinants on HLA-B molecules bearing other HLA-B determinants. This contention was chemically confirmed when Ayres and Cresswell showed that HLA-Bw4 was on the same molecule as HLA-B12 and HLA-Bw6 was on the same molecule as HLA-B7 (31). This study was somewhat limited in scope because all antigenic material was derived from a lymphoblastoid line.

The concept of common antigenic determinants, though relatively recent in the HLA system, has been well established in the homologous mouse H-2 system (32). A survey of antigenic determinants on H-2K and H-2D molecules by haplotype indicates that there are two classes of determinants. Certain determinants are found only in certain haplotypes. Thus, for example, for H-2K molecules, H-2.33 is found only in haplotype *b*, H-2.31 only in haplotype *d*, H-2.23 only in haplotype *k*, and so on. Since each of these determinants is found in only one haplotype, they are known as private determinants. In contrast, other determinants, e.g., H-2.1,3,5,27,28,29, located on the same glycoprotein chain as the private determinants, are found on the H-2K molecules from a number of haplotypes. For this reason, these latter determinants are known as "public" determinants. These public determinants account for the cross-reactivity seen in the H-2 system.

Using the mouse system as a model, we have developed the concept that HLA-B7, Bw22, B27, and B40 are analogous to the murine private determinants, whereas X is analogous to a public determinant. Again, the public determinant accounts for the cross-reactivity.

The various degrees of binding of anti-X to the different X-bearing molecules, as determined by cytotoxicity testing, Protein A binding, and chemical analyses, merit some comment. It seems reasonable to make two assumptions regarding HLA-B molecules on a given cell type. First, the number of HLA-B molecules present on the cell surface is independent of the par-

ticular antigenic determinant borne by those molecules; i.e., for a given cell type, the cell surface density of HLA-B molecules on a B7/B35 cell is identical to that on a B8/B27 cell, which is identical to that on a B7/B27 cell, etc. Second, on a given cell, all HLA-B molecules determined by a single haplotype are identical; i.e., in a B7/B- cell, all B7 molecules are identical, and therefore all bear determinant X.

Given these two assumptions, it follows that the differential binding of anti-X to the various X-bearing molecules on the several cells studied is not because of differences in the number of X-bearing molecules per cell, but is dependent on the particular private determinant borne on the X-bearing molecule. In other words, the private determinant exerts an "allosteric" effect on the public determinant X in so far as it determines the "availability" of X to bind anti-X, and possibly the availability of X to other extrinsic agents (see below).

Because HLA-B antigens belonging to the B7 cross-reactive group have been recently associated with spondyloarthropathies (9, 10), the finding of X as a common determinant on these molecules has implications with regard to both disease predisposition and HLA typing as a diagnostic aid. By far, the strongest associations have been seen with one member of the group, HLA-B27. Although many theories have been put forth to explain the association of spondyloarthropathy with HLA-B27, they can conveniently be divided into two major classes: HLA-B27 directly predisposes the individual to the disease; and the gene encoding HLA-B27 is in strong linkage disequilibrium with a disease susceptibility gene which predisposes the individual to the disease. Clearly, the same two major possibilities are also applicable with respect to the other X-bearing HLA-B molecules. There is at present insufficient data to allow a conclusive determination between these two possibilities. Nonetheless, the very fact that an association exists between the spondyloarthropathies and the X-bearing HLA-B molecules tends in our opinion to favor the direct predisposition hypothesis. One might even postulate that it is the determinant X itself which may be playing a role in predisposition.

A corollary of this postulate would then be that the private HLA-B determinants (i.e., HLA-B7, Bw22, B27, B40, and Bw42) on X-bearing molecules would be found associated with the spondyloarthropathies in frequencies directly proportional to the "availability" of determinant X to the agent presumptively causing these diseases. It is of interest to note in this regard that the frequencies of the association of these private determinants with the spondyloarthropathies rank in a hierarchy which exactly mimics the availability of X as determined by the $^{125}$I-Protein A binding assay, i.e., B27 > B7 > Bw22. (B40 was not found in spondyloarthropathy patients in the two studies cited, and no Bw42 individual was available for our studies.)

With regard to the linked disease susceptibility gene hypothesis, on the other hand, it might be speculated that a primordial HLA-B gene determining X was in strong linkage disequilibrium with a disease susceptibility gene which predisposed the individual to spondyloarthropathy, and that this disequilibrium persisted despite "mutations" leading to "new" private HLA-B determinants on the X-bearing molecule. The mutations, however, would alter the degree of linkage disequilibrium. Thus, the disequilibrium would be highest with the gene determining B27, followed in order by those determining B7, Bw40, and Bw22, thereby yielding the hierarchy of disease associations noted.

Finally, from a practical point of view, since anti-X would detect individuals having any of at least four of the HLA-B antigens belonging to the B7 cross-reactive group, anti-X might prove even more useful than anti-B27 as a diagnostic aid in identifying individuals at risk for the spondyloarthropathies. We have in fact recently initiated such a study, and will present the data in a separate report.

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