EPSTEIN-BARR VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES AS PROBES OF HLA POLYMORPHISM

Heterogeneity of T Cell-restricting Determinants Associated With the Serologically Defined HLA-A2 Antigen*

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Restriction of virus-specific cytotoxic T cell function by products of the major histocompatability complex (MHC),[1] a phenomenon first described in murine systems (1–3), has now been confirmed for the human T cell responses to antigens on cells infected by influenza (4, 5), Epstein-Barr (EB) (6, 7), and more recently, mumps (8), measles (9), and cytomegalo- (10)[2] viruses. In each case the pattern of target cell lysis is consistent with a restriction through class I histocompatability antigens; moreover, monoclonal antibodies to these antigens have been shown to protect target cells both from influenza virus- and EB virus-specific T cell-mediated cytotoxicity (11, 12). One important issue yet to be resolved concerns the relationship between those polymorphisms of HLA antigen structure that serve as restricting determinants for cytotoxic T cell function and those polymorphisms which have for some time been defined by serological techniques, i.e., by "HLA-type-specific" antisera obtained from multiparous women (13).

Studies with influenza virus-specific cytotoxic T lymphocytes first indicated that the above relationship, although close, is not absolute. Thus HLA-A2-restricted effector cells prepared from several different individuals all failed to recognize virus-infected target cells from a particular donor, M7, who was serologically typed as positive for HLA-A2 (14), and it was shown subsequently that M7's HLA-A2 antigen was distinct from the "common" A2 not only in terms of T cell restriction but also in its biochemical characteristics as revealed by isoelectric focusing (15, 16).

Clearly human cytotoxic T cells specific for viruses other than influenza also have the potential ability to distinguish variation in class I HLA antigen structure. Indeed, in the long run, it will be important to determine whether cytotoxic T cells with different viral specificities all reveal the same pattern of HLA poly-

morphism or whether the pattern is itself influenced by the nature of the viral target antigen. The present report describes experiments in which EB virus-specific cytotoxic T cells, prepared from HLA-A2-positive donors and expanded in vitro to give interleukin 2 (IL-2)-dependent T cell lines, have been tested on a large panel of target cell lines of known HLA type. The results on the one hand show that serologically indistinguishable HLA-A2 antigens can indeed bear different T cell-restricting determinants, while on the other hand provide one instance in which a serologically defined cross-reaction between otherwise quite distinct antigens, HLA-A2 and HLA-Bw57, appears to be reflected in a corresponding cross-reactivity of cytotoxic T cell-restricting determinants.

Materials and Methods

Blood Donors. 60-ml blood samples were taken from healthy adult donors by venipuncture into sterile heparinised (10 U/ml) syringes, and peripheral blood mononuclear (PBM) cells were separated by centrifugation on Ficoll-Hypaque. The donors' immune status with respect to EB virus was assessed by measuring serum antibodies to the EB viral capsid antigen (17).

Cell Lines. EB virus-transformed lymphoblastoid cell lines were prepared from blood donors by experimental infection of T lymphocyte-depleted PBM cells (18). The lymphoblastoid cell line from donor M7 was kindly provided by Dr. W. E. Biddison, National Institutes of Health, Bethesda, MD. In addition, certain experiments included the EB virus genome-negative human leukemic cell lines HSB2 and K562, known to be very sensitive to natural killer-like cytotoxicity (19). All the cell lines were maintained by twice-weekly subculture in RPMI 1640 medium supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% fetal calf serum ("culture medium").

HLA-typing. Typing of donors for HLA-A, -B, and -C, and HLA-DR antigens was carried out on PBM cells and on T-depleted PBM cells, respectively; with certain donors the typing was confirmed on the corresponding EB virus-transformed cell line.[3] These tests were kindly performed by Dr. B. A. Bradley and staff of the UK Transplant Service, South West Regional Transfusion Centre, Southmead, Bristol, UK, or in some cases by Dr. A. Ting and staff of the Nuffield Department of Surgery, John Radcliffe Hospital, Oxford, UK.

Immunofluorescence Testing with Monoclonal Antibodies. The monoclonal antibodies used were W6/32 (20), specific for a framework determinant on all HLA-A, -B, and -C antigens (kindly provided by Dr. W. F. Bodmer and Dr. M. J. Crumpton, Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London); MA2.1 (21), specific for a polymorphic determinant on HLA-A2 and on HLA-B17 (Bw57/58) antigens (kindly provided by Professor A. J. McMichael, John Radcliffe Hospital); and FMC5 (22), specific for a polymorphic determinant on HLA-A2 (kindly provided by Dr. H. Zola, Department of Clinical Immunology, Flinders Medical Centre, Bedord Park, South Australia). Indirect immunofluorescence testing of lymphoblastoid cell lines for expression of HLA-A2 was performed exactly as described elsewhere (23): W6/32 served as a positive control antibody and each test included a number of cell lines negative for HLA-A2 by conventional typing tests.

Preparation of EB Virus-specific Effector T Cells. EB virus-specific cytotoxic T cell precursors, present in the blood of immune donors, were reactivated in vitro using a modified version of published methods (24, 25). Briefly, 2 × 10⁶ PBM cells were cultured with 5 × 10⁴ x-irradiated (4,000 rad) stimulator cells of the autologous EB virus-transformed cell line in 2-ml flat-bottomed wells (Linbro Chemical Co., Hamden, CT). After 10 d incubation at 37°C, in an atmosphere of 5% CO_2 in air, the cultures were harvested and the T cell fraction prepared by E-rosetting with sheep erythrocytes treated with 2-aminoethyli-

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sothiouronium bromide hydrobromide. A second in vitro stimulation was then carried out by culturing 5×10^5 T cells with 2×10^5 x-irradiated stimulator cells in 2-ml wells for a further 4–6 d, after which 5×10^5 of the viable cells from these cultures were reseeded with 2×10^5 x-irradiated stimulator cells in the presence of culture supernatants that contained IL-2 prepared as described elsewhere (25). Cytotoxic T cell lines established in this way could be maintained for many weeks by regular reseeding in IL-2 medium (twice weekly) with weekly additions of x-irradiated stimulator cells.

Cytotoxicity Assays.   Effector T cells from the IL-2-dependent T cell lines were washed and resuspended in fresh IL-2-free medium for 12–18 h before assay. Target cells and where necessary unlabelled "cold targets," were taken from the panel of cell lines described above; on certain occasions, target cells included mitogen-stimulated lymphoblasts prepared from cultures of PBM cells either 3 d after exposure to phytohemagglutinin (PHA) or 5 d after exposure to pokeweed mitogen (PWM). The conduct of the 5-h chromium-release assay, and of cold target competition experiments was exactly as described elsewhere (26), except that the majority of assays were conducted over a range of different effector/target ratios (2.5:1 to 20:1).

Monoclonal Antibody Blocking of Cytotoxicity.   Target cells were pretreated with high concentrations of monoclonal antibodies W6/32 and MA2.1 (W6/32 at a final dilution of 1:200 and MA2.1 at a final dilution of 1:20–1:40 of purified immunoglobulin preparations) for 20 min at 37°C in the assay wells before addition of effector cells. In all tests some target cells were similarly treated but not exposed to effector cells, to confirm that the antibodies were not cytotoxic per se. As a further control, high concentrations of monoclonal antibodies directed towards non-HLA structures on the target cell surface (23) were included in certain assays, and never affected the level of lysis.

Analysis of Results of Cytotoxicity Assays.   To allow comparison of results from repeated testing of the same effector/target cell combination, the specific lysis was expressed on each occasion as a percentage of the autologous target cell lysis observed in the same experiment at the same effector/target ratio. The mean and standard deviation of the relative percentage lysis were then calculated for each effector/target combination. In the present experiments, the absolute level of autologous target cell lysis seen in the 5-h chromium-release assay was usually between 35 and 55% at an effector/target ratio of 5:1.

Experimental Procedure.   EB virus-specific cytotoxic T cell lines were established from nine virus-immune donors positive for the serologically defined HLA-A2 antigen, and the identity of the dominant class I HLA-restricting determinants was revealed by testing on a suitable panel of HLA-typed lymphoblastoid cell lines. Effector T cells lines that possessed demonstrable A2-restricted cytotoxicity (from four of the original nine donors) were selected for further study. Each was tested against a common panel of 20 EB virus-transformed HLA-A2-positive target cell lines, including in each case the autologous line as well as a majority of the available lines matched with the effector cells through HLA-A2 alone. Matching through HLA-C and -DR antigens was not considered since these molecules do not serve as major restricting determinants of EB virus-specific cytotoxicity (12, 26).

In further experiments, lysis of autologous and of HLA-A2-matched target cells by all four populations of effector cells was tested in the presence and absence of high concentrations of the monoclonal antibodies MA2.1 and W6/32. The specificity of one population of effector cells was further examined by testing on both EB virus-transformed and mitogen-stimulated target cells from the same individuals, as well as in cold target competition experiments.

Results

HLA-restricted Nature of EB Virus-specific Cytotoxic T Cell Lines.   Fig. 1 presents the pattern of target cell lysis shown by the cytotoxic T cell line established from EB virus-immune donor BW, the results having been compiled from between two and six separate tests of each effector/target combination with standard
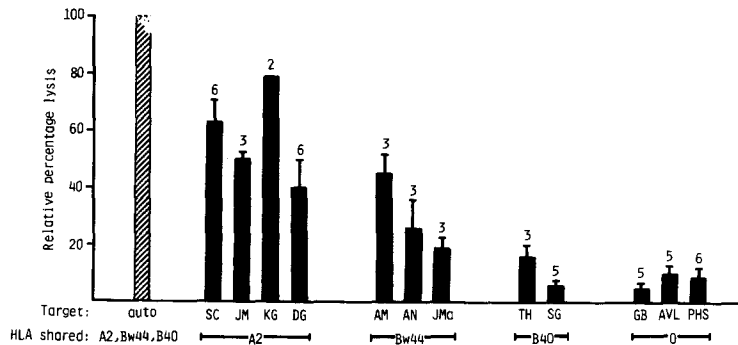
FIGURE 1. Cytotoxicity of an effector T cell line from donor BW (A2, B40, Bw44) on a range of target cell lines whose degree of HLA-A and -B antigen matching with the effector cells is as shown. The relative percentage lysis shown for each target represents the specific lysis of that target cell line expressed as a percentage of the specific lysis of the autologous (BW) target cell line obtained in the same assay at the same effector/target cell ratio. The numbers above each column denote the number of occasions on which each target was tested, with the standard deviations as shown.

TABLE I

Dominant HLA Antigens Restricting Cytotoxicity of EB Virus-specific T Cell Lines

Donor		HLA type			Dominant HLA antigens restricting cytotoxicity
BW	A2	B40,w44	Cw3,w5	DR4	A2, Bw44
KG	A2	B8,14	C−	DR3,7	A2, B8
DG	A2,28	B8,18	C−	DR3,4	A2, B8
EL	A2	B37,w44	Cw5,w6	DR4	A2, Bw44

deviation as shown. The figure shows only a representative range of the target cell lines actually tested. Although the autologous line was always perferentially killed, lysis of allogeneic EB virus-transformed lines could clearly be mediated through HLA-A2 antigen matching and to a lesser extent through Bw44 antigen matching. In contrast, target cells lines bearing only B40 in common with the effector cells were not killed above the low background level shown by HLA-mismatched lines. This is a reflection of a more general phenomenon seen in this system whereby effector cell preparations from individual donors consistently show preferential restriction through particular HLA-A or -B antigens (26). Thus, of nine HLA-A2 EB virus-immune donors tested in the course of this work (five of whom are A2 homozygous), only four (three of whom are A2 homozygotes) generated cytotoxic T cell lines containing a demonstrable A2-restricted component.

Table I gives the HLA types of these four donors and indicates in each case the HLA antigens which were dominant restriction elements (as determined from tests upon a panel of 30–50 allogeneic virus-transformed cell lines of known HLA type). It is noteworthy that these effector T cell preparations, like those described in earlier work (26), did not kill the EB virus-genome-negative leukemic cell lines HSB2 and K562, which were included as sensitive indicators of nonspecific natural killer-like activity (data not shown).

Failure of HLA-A2-restricted Cytotoxic T Cells to Lyse a Subgroup of A2-positive Targets.   Fig. 2 presents the composite results of those assays in which effector T cells from donors BW, DG, KG, and EL were each tested on the same panel of 20 EB virus-transformed target cell lines all bearing the serologically defined HLA-A2 antigen. For each effector/target combination, the level of lysis shown (expressed as a percentage of the autologous cell line lysis seen with the same effector cells) represents the mean of between 2 and 10 separate assays. Most targets in the panel shared only HLA-A2 with the effector cells, but those sharing other HLA-A or -B antigens are indicated on the figure; the autologous cell line lysis is shown by a hatched column in each case.

It is clear that 16 of 20 HLA-A2 target cell lines were consistently lysed by all four effector populations at levels above 25% of autologous cell lysis. In contrast, there was no significant killing of three particular lines (AMc, AD, M7) by any of the effector cell populations, nor of one line (RT) by three of the four effector T cell lines. The discordant results obtained with these target cell lines were consistent in repeated testing of each of the effector cell populations and were confirmed when new cytotoxic T cell preparations were made from fresh blood samples from two available donors (KG, DG).

The distinctions between different cell lines within the target cell panel apparent from Fig. 2 were clearly seen across a range of effector/target cell ratios as exemplified by the results of Fig. 3. Increasing numbers of effector cells from donor BW showed increasingly strong lysis of five HLA-A2-matched target cell lines from the main panel, while lysis of the AMc, RT, and M7 cell lines remained at the very low level shown with an HLA-mismatched control target. Such results did not reflect an insensitivity of these particular cell lines to lysis by all EB virus-specific effector cells, since in parallel tests each of these lines has been reproducibly killed by virus-specific effector cell populations restricted through antigens other than HLA-A2. Thus RT cells are sensitive to A3- and B7-restricted effectors and M7 cells are sensitive to Bw35-restricted effectors (data not shown).

Blocking of HLA-A2-restricted Lysis by an A2-specific Monoclonal Antibody.   Fig. 4 shows representative data from further experiments in which high titer preparations of the monoclonal antibodies MA2.1 (anti-HLA-A2) and W6/32 (anti-A, -B, -C common antigen) were tested for their effect upon target cell lysis. These particular results were obtained using effector cells from donor DG but illustrate a pattern that was observed for all four effector cell preparations. Thus MA2.1 had only a marginal effect upon autologous target cell lysis when compared with the marked reduction of this lysis seen in the presence of W6/32. However, where the effector and target cells were matched only through HLA-A2 (targets EL and AH), MA2.1 blocked lysis as effectively, as did W6/32. Moreover the HLA-A2-specific monoclonal antibody had no effect on the HLA-B8-restricted cytotoxicity present within this same effector population (target LGYS). It was interesting to note that the low level of killing of the anomalous HLA-A2-positive target cell line M7 was also unaffected by MA2.1.

Cross-reactive Lysis of HLA-Bw57-positive EB Virus-transformed Cells by Effector Cells from Donor EL.   The pattern of results obtained with effector cells from donor EL on the panel of HLA-A2-matched target lines was essentially similar to that
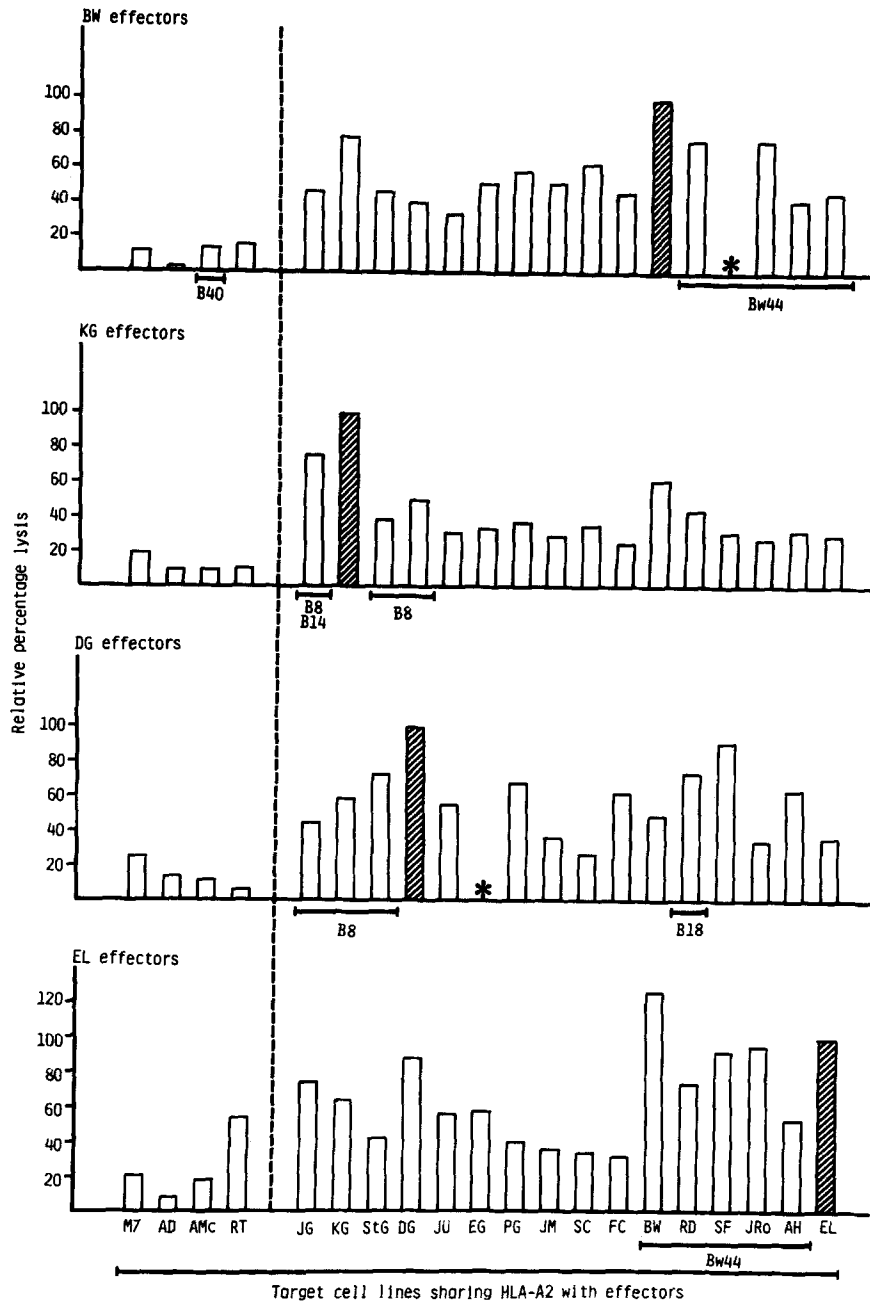
FIGURE 2.   Cytotoxicity of effector T cell lines from four HLA-A2-positive donors (BW, KG, DG, EL) on a common panel of 20 target cell lines all expressing the serologically defined HLA-A2 antigen. The autologous target cell line for each effector cell population is denoted by the hatched bar; where particular effector/target cell combinations involve HLA-A or -B antigen sharing in addition to HLA-A2 sharing, this is indicated under the relevant result. For each target cell line, the results are expressed as relative percentage lysis, as described in Fig. 1 legend. (*) indicates not tested.
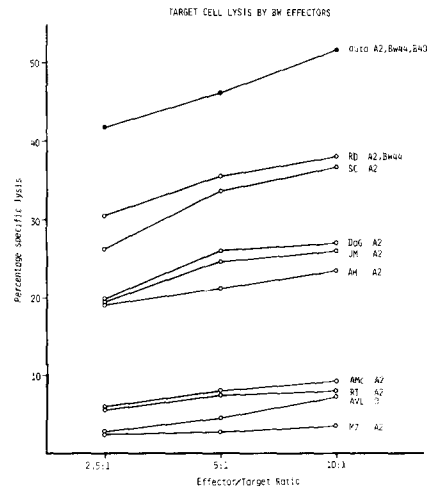
FIGURE 3. Cytotoxicity of an effector T cell line from donor BW (A2, B40, Bw44) on a range of target cell lines tested at various effector/target cell ratios. The results are expressed as percentage specific lysis of the autologous target cell line (●) and of various allogeneic target cell lines (○) whose degree of HLA-A and -B antigen matching with the effector cells is as shown.
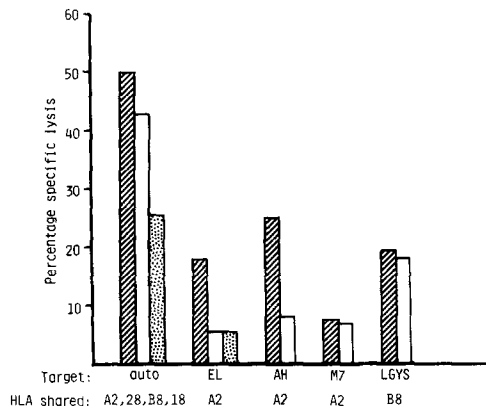

FIGURE 4. Effect of monoclonal antibodies on the level of lysis of autologous, HLA-A2 antigen-matched and HLA-B8 antigen-matched target cell lines by effector T cells from donor DG (A2, A28, B8, B18). Results are expressed as percentage specific lysis in the absence of antibody (hatched bar), in the presence of saturating concentrations of monoclonal antibody MA2.1 (open bar), or of monoclonal antibody W6/32 (dotted bar).

shown by the other three effector cell populations except that EL effector cells showed consistent lysis of the otherwise anomalous RT cell line (Fig. 2). It was therefore most important to determine whether this did indeed represent virus-specific HLA-A2-restricted killing. In this regard, it was found that lysis of RT cells by EL effector cells was very sensitive to the blocking effect of the monoclonal antibody MA2.1 (>80% reduction); lysis of other HLA-A2-positive targets such as DG cells by the same effector cells was less sensitive to blocking (30–40% reduction) in these particular tests. Lysis of both RT and DG cells proved equally sensitive to blocking by W6/32 (30–40% reduction) in these same tests.

This result raised the possibility that lysis of RT cells was mediated through a polymorphic HLA determinant closely associated with the MA2.1 binding site and present on the HLA-A2,A3,B7,Bw57-positive RT cell line. The fact that MA2.1 binds not only to HLA-A2 antigens but also to a cross-reactive epitope on HLA-Bw57 antigens (21) prompted a final series of experiments in which the testing of EL effector cells was extended to include HLA-A2-negative, Bw57-positive target cell lines.

The results, presented in Fig. 5A, clearly show the existence of a cross-reaction against Bw57-positive cell lines LM and TH, which, though not quite as strong as the well-documented reaction against A2-positive lines such as DG and KG, was nevertheless significant and reproducible. In contrast, mitogen-stimulated lymphoblasts from donors RT and LM (Fig. 5A) as well as from other Bw57-positive donors (data not shown) did not suffer significant lysis when included as targets in the same assays. Finally, in cold target competition experiments designed to identify the dominant antigen restricting the EL/RT effector/target interaction, lysis of the RT cell line was inhibited very efficiently by the A2-negative, Bw57-positive TH cell line, whereas the A2-positive, Bw57-negative DG and KG cell lines competed only marginally better than two negative controls (Fig. 5B). Furthermore, the HLA-A2-restricted lysis of the KG cell line by EL effector cells could be inhibited by another HLA-A2-positive cell line (JM) from the main HLA-A2 panel, while the RT cell line itself proved to be a relatively poor competitor (Fig. 5C).

Discussion

The evidence is now very strong that class I HLA antigens on the surface of target cells bear the polymorphic restricting determinants that govern virus-specific cytotoxic T cell function in man (4–10, 27).[2] However, it is not yet clear how closely the serologically defined polymorphisms of HLA antigen structure reflect the heterogeneity of T cell-restricting determinants or how this heterogeneity might itself be influenced by the identity of the viral antigen against which the cellular response is directed. The present work describes experiments in the EB virus system that bear principally upon the first question but which could in the longer term contribute towards resolution of the second.

The present study was made possible through the development of methods whereby EB virus-specific cytotoxic T cells, reactivated in vitro from memory cells in the circulation of virus-immune individuals (18), could be expanded as IL-2-dependent effector cell lines (25). These lines displayed a virus-specific, HLA-restricted cytotoxicity in which the pattern of restriction of the original effector cell population (often involving preferential recognition through just one or two of the available HLA-A and -B antigens) was faithfully maintained. The present studies focused on cytoxicity through HLA-A2, since experiments with influenza virus-specific cytotoxic T cells had already suggested the existence of heterogeneity within this serologically defined antigen (14–16, 28). Out of nine HLA-A2-positive EB virus-immune donors from whom effector cell lines were established, only four (including three A2 homozygotes) generated a demonstrable A2-restricted component within the polyclonal, cytotoxic response (Table I). Since the five donors with no detectable A2-linked response were all
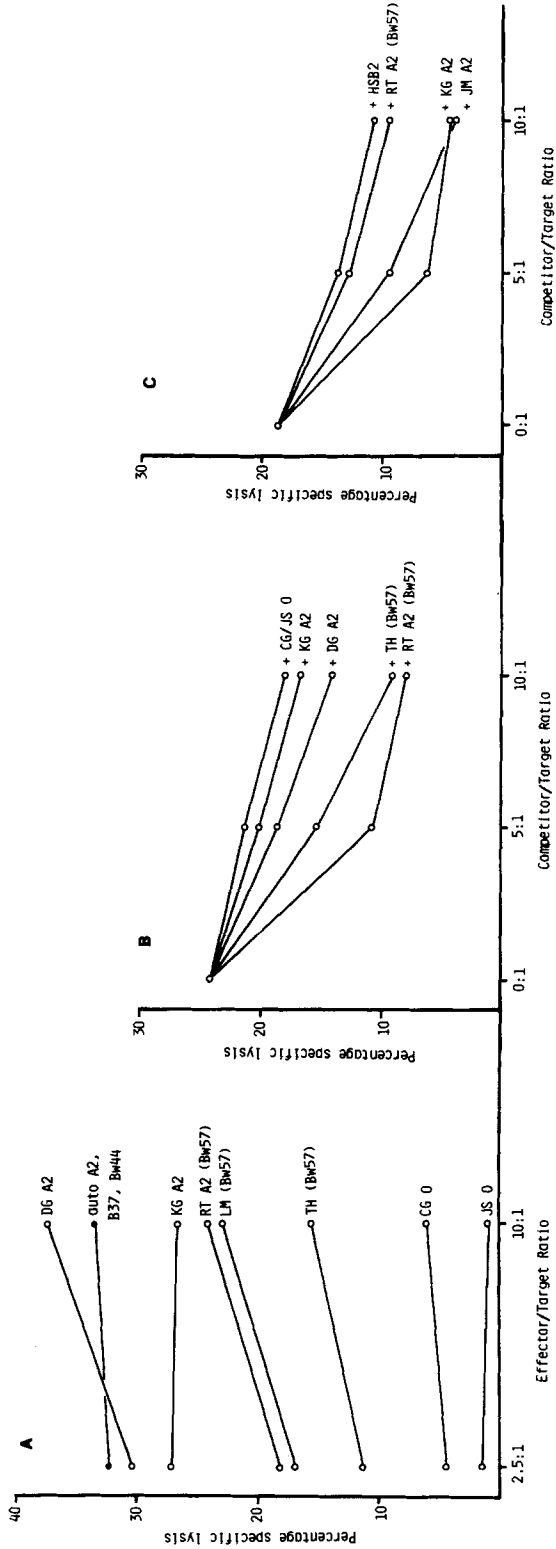
FIGURE 5.   (A) Cytotoxicity of an effector T cell line from donor EL (A2, B37, Bw44) tested at two effector/target cell ratios on the autologous target cell line (●) and on various allogeneic target cell lines (○) whose expression of HLA-A2 and of HLA-Bw57 antigens is as shown. Using the same effector T cells at the same effector/target ratios, levels of specific lysis of mitogen-stimulated lymphoblasts were as follows: RT lymphoblasts, 1.5 and 4.5%; LM lymphoblasts, −2.1 and −0.6%. (B) Inhibition of lysis of target cell line RT by cold target competitor cell lines whose expression of HLA-A2 and of HLA-Bw57 antigens is as shown. Effector T cells from donor EL (A2, B37, Bw44) were used at an effector/target ratio of 5:1, both in the absence of competitor cells and at competitor/target ratios of 5:1 and 10:1, as shown. (C) Inhibition of lysis of target cell line KG by cold target competitor cell lines whose expression of HLA-A2, and HLA-A2 and HLA-Bw57 antigens is as shown. Effector T cells from donor EL (A2, B37, Bw44) were used at an effector/target ratio of 5:1, both in the absence of competitor cells and at competitor/target ratios of 5:1 and 10:1, as shown.

subsequently shown (through testing of their virus-transformed cells as targets) to bear the "common A2" antigen and not some unusual variant, the results in these cases are consistent with our earlier observations (26) suggesting that A2 is not usually a preferred antigen for the restriction of EB virus-specific cytolysis.

Testing of four separate HLA-A2-restricted effector cell populations on the same panel of 20 HLA-A2-positive EB virus-transformed target cell lines clearly identified a group of four lines (RT, AMc, AD, M7) whose lysis was generally very low. When the cells of these particular lines were serologically typed, their HLA antigen expression was in accord with that expected from the HLA type of the original donor and included expression of the serologically defined HLA-A2 molecule. Moreover, the four lines, as well as a representative number of lines tested from within the main panel of target cells, all bound the HLA-A2/B17-specific monoclonal antibody MA2.1 and the monoclonal antibody FMC5, whose reactivity, though less well defined, is also against an HLA-A2-associated determinant. The discordant results obtained with the RT, AMc, AD, and M7 target cell lines in the cytotoxicity assays in Figs. 2 and 3 did not reflect an insusceptibility to EB virus-specific cytolysis per se since each line could be killed by virus-specific effector cells restricted through other class I HLA antigens on the target cell surface. The A2-restricted nature of the killing of those target cell lines within the main body of the panel was confirmed, in that lysis could be specifically blocked by high concentrations of the MA2.1 monoclonal antibody. In these same tests, the much lower level of killing directed against the anomalous target cell lines was not at all affected by MA2.1, suggesting that none of this residual lysis was actually HLA-A2 restricted.

The inference from these results must be that EB virus-specific cytolysis was being mediated through a polymorphic determinant (or determinants) common to most, but not all, serologically defined HLA-A2 molecules. It was interesting to note that, even within the panel of common-A2–bearing targets, each effector cell population produced its own characteristic pattern of lysis, with stronger reactivity against some targets than others. This is somewhat reminiscent of results recently obtained from an analysis of HLA-A3-restricted T cell cytolysis in the influenza virus system (29) that led those workers to suggest the existence of multiple restriction sites on the A3 molecule. A similar inference might also be drawn for the common A2 molecule from the present work, but positive proof of this thesis requires evidence from A2-restricted T cell clones, each specific for a single restriction site.

It will be important to determine whether the four target cell lines RT, AMc, AD, and M7, serologically typed as HLA-A2-bearing but lacking the common-A2–associated restricting determinant(s), all possess the same variant A2 molecule or whether each is itself distinct; the latter suggestion may be more likely because donors RT and AMc are Caucasian in origin, while M7 and AD are Black and Asian, respectively.

In this context, the consistent lysis of RT target cells by effector cells from donor EL was particularly interesting, especially as EL is homozygous for HLA-A2 (serologically defined) and might conceivably possess T cells restricted through a "variant A2"–associated determinant in addition to the common-A2–associated reactivity dominating the other three effector cell preparations tested

in this work. In fact, further analysis of the effector EL/target RT interaction did not substantiate this possibility but instead revealed the existence within the EL effector cell population of an unexpected cross-reactivity directed against HLA-Bw57-bearing target cell lines (Fig. 5).

This was not a cross-reactivity against an allo-determinant per se, of the kind that has been detected in various antigen-specific murine cytotoxic T cell systems (30–32), since only EB virus-transformed and not mitogen-stimulated (PHA or PWM) Bw57-bearing lymphoblasts were recognized. Thus it appeared to reflect a cross-reactivity at the level of HLA-restricting determinants that mediated EB virus-specific cytolysis. In relation to this, it is important to note that HLA-A2 (the dominant restricting element for EL effector cell preparations) and HLA-Bw57 (the source of cross-reactivity) are already known to share common epitopes as defined by the monoclonal antibody MA2.1 (21) and by certain conventional HLA typing sera (33). The fact that MA2.1 blocked the effector EL/target RT interaction with such unusual efficiency adds weight to the view that lysis here is being mediated through a restricting determinant common to HLA-A2 and Bw57 molecules. If this is the case, clearly only a minority of the A2-restricted clones within the EL effector cell population recognize this particular determinant, since lines bearing the common A2 antigen such as DG or KG proved relatively poor competitors of RT cell lysis in comparison with HLA-Bw57-bearing competitor cell lines (Fig. 5B). Although the point is by no means proven, these findings do highlight the possibility that known, serologically defined cross-reactions between otherwise distinct HLA antigens might in some cases identify common epitopes capable of restricting T cell function. The possibility is one that must now be pursued by T cell cloning.

Finally, the present work must be viewed in the context of parallel studies in the influenza virus-specific (14–16, 28), H-Y antigen-specific (34, 35), and allo-specific (36, 37) human cytotoxic T cell systems, each of which also provides evidence of HLA-A2 heterogeneity. It will be important to compare the subdivisions of HLA-A2 that these different cellular probes reveal. The limited evidence available to date on this point is interesting in that, of the donors with variant A2 expression identified in this present study using EB virus-specific effectors, both M7 (14, 15, 34) and AMc (28, 35; referred to as AM) have also been detected as A2 variants using influenza virus-specific and H-Y antigen-specific cytotoxic T cells. It remains to be seen whether this pattern of agreement is maintained with more extensive analysis and indeed whether heterogeneity of HLA-A2 as defined by cellular probes is always reflected in altered biochemical properties of the A2 molecule (16). Whatever the answers to these questions, the work on HLA-A2 antigen heterogeneity has clearly established an experimental basis for the more general analysis of polymorphism amongst MHC gene product in man.

Summary

Epstein-Barr (EB) virus-specific effector T cell lines were established from nine virus-immune donors positive for the serologically defined HLA-A2 antigen; of these, four lines contained a demonstrable A2-restricted cytotoxic component. When these four effector populations were each tested on the same panel of EB

virus-transformed lines from 20 HLA-A2-positive individuals, 16 of the target cell lines were consistently killed at levels above 25% of the relevant autologous cell lysis. Cytotoxicity appeared to be mediated through a restricting determinant associated with the 'common A2' antigen that these lines shared; indeed the lysis could be specifically blocked by high concentrations of an HLA-A2-specific monoclonal antibody. In contrast, 4 out of 20 target cell lines were not killed by HLA-A2-restricted effector cells, even though they did express the serologically defined A2 antigen and were found in other tests to be susceptible to EB virus-specific cytolysis restricted through other HLA-A or -B antigens on their surface.

These results suggest that EB virus-specific cytotoxic T cells can distinguish between serologically identical HLA-A2 molecules via the heterogeneity of their T cell-restricting determinants. Data from one of the effector cell populations further suggested that a serologically defined cross-reaction between the otherwise distinct HLA-A2 and -Bw57 antigens might also be reflected in a cross-reactivity of T cell-restricting determinants.

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