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Human Leukocyte Antigen-Linked Genetic Controls for T Cell-Mediated Cytotoxic Response to Mumps Virus in Humans

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The involvement of human leukocyte antigen determinants for the response of mumps virus-specific cytotoxic T lymphocytes in humans was studied. The cytotoxic T lymphocytes could only lyse virus-infected allogeneic cells with which they shared a particular human leukocyte antigen, e.g., Bw52 or B7. The existence of Bw52 in the subjects who received a booster immunization with live mumps vaccine was associated with a significantly higher cytotoxic T-lymphocyte response than that of subjects without the gene. Although a donor-dependent difference in the recognition of human leukocyte antigen-A2 suggests the complexity of the genetic mechanisms involved, the results are largely consistent with the concept of major histocompatibility complex-linked genetic control of virus-specific cytotoxic T-lymphocyte response.

Recent studies of the virus-specific cytotoxic T-lymphocyte (CTL) response of mice have demonstrated that the genes of the major histocompatability complex (MHC) can, by and large, exert two kinds of controls on the response. Thus, the CTLs can only lyse virus-infected target cells with which they share the same products of the *H-2K* or *H-2D* region or of both regions of the MHC (5). In addition to coding these self-restricting antigens, MHC-linked genes can control the magnitude of such antigen-specific and MHC-restricted CTL responses (4, 14). Similar phenomena have also been revealed in CTL response to influenza viruses in humans (2, 7, 9, 10) in that the response has been shown to be controlled by genes closely linked to human leukocyte antigen (HLA)-A and -B loci. Studies for CTLs against Epstein-Barr virus also suggest the existence of such HLA-related controls, although the restriction pattern is less clearly defined (6, 8). Since MHC-restricted CTL responses to virus have been suspected to play a major role in preferential host survival in mice (13), it is clearly important to investigate further the relationships between the responses of CTLs specific for other viruses and polymorphic HLA determinants in humans also.

Previously, we devised a method for in vitro generation of mumps virus-specific CTLs and showed that the assay can be used to reflect T cell-mediated immunity in humans (11). Subsequently, we have studied the involvement of the HLA-A and -B genes in the responses among a population of immune adults.

MATERIALS AND METHODS

Virus. Egg-grown mumps virus (Enders strain) was partially purified by centrifugation, as previously described (11). The virus preparation had 512 to 1,024 hemagglutination units per 0.025 ml and was stored at −80°C until needed.

Lymphocyte donors and vaccination. Heparinized peripheral blood was obtained from healthy immune adults and from those who received a vaccination of live mumps vaccine (3×10^6 to 5×10^6 PFU of the Urabe strain, kindly supplied by Michiaki Takahashi, Research Institute for Microbial Disease, Osaka University, Osaka, Japan) (12). HLA-A and -B locus typing of these donors had been performed by the standard National Institutes of Health technique.

Preparation of effector and target cells. Procedures for preparation of effector and target cells have been described in detail previously (11). Briefly, fresh lymphocytes were separated from a single specimen of blood by flotation on Ficoll-Hypaque and were suspended at 2×10^6 per ml of RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 60 μg of kanamycin sulfate per ml, 20% fetal calf serum, and 20 mM HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). For preparation of effector cells, 5 ml of the suspension was cultured in plastic flasks (no. 3012; Falcon Plastics, Oxnard, Calif.) in the presence of UV-inactivated mumps virus (4,096 hemagglutination units; 0.1 to 0.2 ml of the stock virus) in an incubator with an atmosphere of 5% CO_2 at 37°C. After 7 days, the number of viable cells was determined by trypan blue exclusion and cells were suspended at 4×10^6 per ml

of assay medium (RPMI 1640 medium with 10% fetal calf serum). Target cells were prepared from the original lymphocyte suspension; they were maintained in RPMI 1640 growth medium for 3 days and were subsequently stimulated by phytohemagglutinin (10 μg/ml; Wellcome Research Laboratories, Beckenham, England) for 4 days. The lymphoblasts were incubated simultaneously with 200 μCi of $Na_2{}^{51}CrO_4$ (The Radiochemical Centre, Amersham, England) and 12,000 hemagglutination units of live mumps virus (0.3 to 0.6 ml of the stock virus) per 2×10^6 to 4×10^6 cells at 37°C for 1 h. The cells were then washed and suspended in assay medium at 10^5/ml.

Cytotoxicity assay. The cytotoxicity assay was performed by a standard method (1). A 100-μl portion of the target cell suspension (10^4 per well) and the same volume of effector cell suspension (4×10^5 per well) were placed in triplicate in a round-bottomed microculture plate (Linbro Plastics, New Haven, Conn.); the plate was then centrifuged at $250 \times g$ for 3 min. After incubation for 4 h at 37°C, the radioactivity in 100 μl of the supernatant was counted, and the percent specific lysis was calculated, as previously described (11). Statistical analysis was performed by Student's t test.

RESULTS

Nonspecific cytotoxic activity of mumps CTLs. Before investigation of the HLA-restricted responses of mumps CTLs, the nonspecific activity of the CTLs on allogeneic target cells were evaluated. Responses on uninfected allogeneic cells did not exceed levels of cytolysis on uninfected autologous cells (Table 1), except in a rare situation in which the cytolysis on infected allogeneic cells was higher than that on infected autologous cells (donor 4). This indicates that the virus-specific component of the response is independent of a particular allogeneic combination of effector and target cells.

Selective recognition of HLA-linked determinants by mumps virus-specific CTLs. First, the cross-reactivity of mumps CTLs on allogeneic target cells totally mismatched for HLA-A and -B antigens was evaluated. These CTLs from six unrelated donors, with or without vaccination, caused 11 to 47% specific ^{51}Cr release against the corresponding autologous target cells, whereas 0 to 5%, which comprised only 6.0 ± 5.9% of the cytolysis on the autologous cells, was observed with a total of 10 such allogeneic cells (data not shown). Of eight combinations in which CTLs shared HLA-A9 with target cells (Fig. 1A), the relative response on these allogeneic cells was 6.9 ± 7.9% of the cytolysis on the autologous cells and did not significantly differ from that of the HLA-mismatched group. Similar results were obtained from other selected combinations of effector and target cells, such as A10, Bw54, B12, Aw33, and Bw16 (Fig. 1B).

In contrast, lysis equivalent to that on the autologous cells was observed when CTLs shared HLA-Bw52 or -B7 with allogeneic target cells (Fig. 1C). Apparently A9 antigen, which is in strong linkage disequilibrium with Bw52, is not a determinant involved in this preferential responsiveness (Fig. 1A). The relative lysis by CTLs on these Bw52-shared allogeneic cells was 104.7 ± 15.3% and was significantly higher than that of the HLA-mismatched combination ($P < 0.001$).

Differential responsiveness of donor to virus in conjunction with HLA-A2. Subsequent analysis for A2-associated responses has revealed that one donor exhibited peculiar reactivity against the antigen (Fig. 1D). Thus, the lysis of donor 1's cells on A2-matched target cells from three unrelated subjects was consistently around 50%

TABLE 1. Cytotoxic activity of mumps CTLs on virus-infected and -uninfected cells

Effector cell donor	Target cell donor	HLA shared[a]	% Specific lysis[b]	
			Infected cells	Uninfected cells
1	Autologous		29.0	5.8
	5	A2	12.5	−2.7
	6	A2	9.0	−1.5
2	Autologous		18.0	2.7
	7	A9	3.1	2.5
	8	A9, w31	3.3	4.1
3	Autologous		14.5	4.6
	2	A9, Bw52	17.3	3.4
	4	A9, Bw52	13.3	2.4
4	Autologous		17.6	3.3
	2	A9, Bw52	27.4	7.4
	3	A9, Bw52	25.9	6.5

[a] HLA types of these donors are given in the legend to Fig. 1.
[b] A target cell/effector cell ratio of 1:40 was used uniformly.

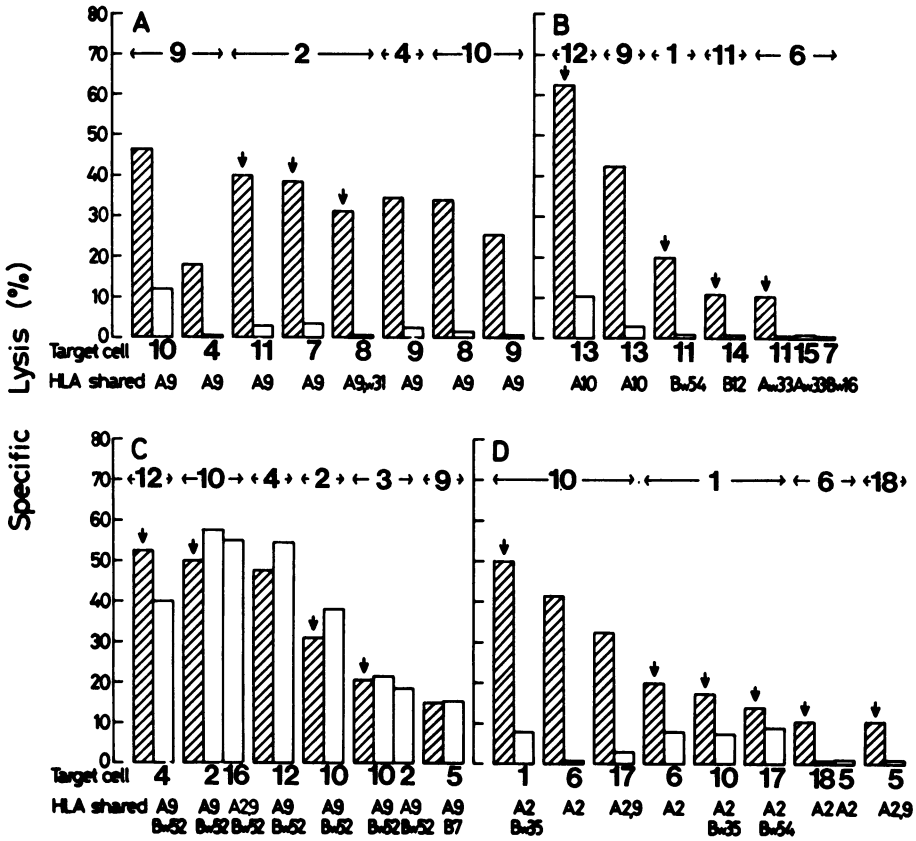

FIG. 1. Specific lysis of autologous (striped bars) and allogeneic (open bars) target cells by mumps-immune cytotoxic T cells. A target cell/effector cell ratio of 1:40 was used uniformly. Donors of effector cells are indicated above the columns, with arrows indicating effector cells obtained after immunization with live mumps vaccine. Target cells and shared HLA antigens with effector cells are indicated below the columns. HLA types of these donors are as follows: 9—A9, A10, B7, –; 2—A9, w31, Bw52, w22; 4—A2, A9, Bw52, Bw40; 10—A2, A9, Bw52, w35; 11—A9, w33, B12, w54; 7—A9, A11, Bw51, w16; 8—A9, w31, Bw51, Bw40; 12—A9, A10, Bw52, w59; 1—A2, A10, Bw35, w54; 6—A2, w33, B17, w16; 13—A10, –, Bw16, B15; 14—A3, A10, B12, B15; 15—Aw33, –, Bw51, w52; 3—A9, –, Bw51, w52, 16—A2, A9, Bw52, –; 5—A2, A9, B7, –; 17—A2, A9, Bw54, B15; 18—A2, A9, Bw51, w59.

of that on autologous cells in three separate experiments. This reactivity did not seem to be due to unusually high natural killer (NK) activity in the effector population because the donor failed to lyse other target cells that were HLA partially matched (Fig. 1B) or totally mismatched. Antigen Bw35, shared with target cells, is not likely to be involved in this reactivity, as suggested by the minimum contribution of this antigen to cytolysis (Fig. 1D). The results also suggest that this cross-reactivity is mediated at the effector cell level since the allogeneic target cells (donors 6 and 17) were not lysed by another A2-possessing donor (donor 10).

Higher response of mumps CTLs in association with HLA-Bw52. Due to relatively low CTL responses in a considerable number of normal immune subjects, attempts have been made to raise the response by booster immunization of the donors with live mumps vaccine. This exceptional procedure also allowed us to evaluate the precise temporal nature of the CTL response in conjunction with selected HLA-A or -B antigens. The maximum responses of donors who possessed Bw52 were consistently higher than those of donors without the antigen ($P < 0.05$), indicating the possibility that the magnitude of mumps CTL response is also controlled by genes closely linked to HLA (Fig. 2).

DISCUSSION

Although a relatively small group of unrelated donors was evaluated in this study, the results for the response of mumps CTLs are generally similar to those of the MHC-restricted CTL responses of mice to virus-infected target cells

(5) and to those of CTL responses to influenza viruses in humans (2, 7, 9, 10). Study of type A influenza virus in members of a large family has clearly demonstrated a close association of genes coding HLA-A and -B antigens with genes coding for the major determinants recognized by virus-specific CTLs (9). Therefore, the present observations for mumps CTLs in which the recognition of HLA determinants has strict selectivity for particular genes, i.e., Bw52 and possibly B7, strongly suggest the existence of similar MHC-linked genetic mechanisms in the control of the response.

In our previous report (11), we described the general characteristics of this in vitro CTL response to mumps and showed that the method can be used for the quantitative estimation of individual responses. Therefore, the difference in the magnitude of the CTL response observed between the two groups of vaccinated subjects, who either are positive for Bw52 or lack the gene, most likely reflects individual characteristics which are in some way under the influence of the gene. Although the precise mechanisms of such phenomena are mostly unknown, several possibilities may be considered. First, the preferential responsiveness associated with the particular gene and not with others may be attributable to the ability of each HLA molecule to form an immunogenic complex with virus antigen (5). The second possibility is that such a difference may be due to a function of genes other than the HLA-A or -B locus, particularly those of the D region which are in strong linkage disequilibrium with structural genes. Although this concept is not necessarily contradictory to the first possibility, studies on the effect of the HLA-D region would be important for evaluation of these two possible mechanisms and are currently being carried out among volunteers of immune adults. An alternative, though less likely, idea is that within the region encoding a particular HLA molecule, e.g., Bw52, there might be a separate gene which controls the magnitude of the response. Although the HLA-D region has been considered to be the human equivalent of the murine *H-21* region, a possible regulatory gene has been demonstrated in the HLA-A locus in the CTL response to Epstein-Barr virus (6). However, in a situation in which the restriction antigen cannot be distinguished from an element which controls the magnitude, it may not be necessary to consider the existence of such a distinct regulatory gene in the same region.

Biddison and Shaw have pointed out in a population study that there is a diversity in the CTL response to HLA-A2 or -B7 in conjunction with influenza viruses, suggesting complexity of genetic mechanisms controlling the response (2, 10). In fact, a similar donor-dependent difference was also observed in the recognition of A2 antigen by mumps CTLs. It has previously been revealed that the murine CTL response to *H-2D^b* specific for certain viruses is regulated by *H-2K* genes (4, 14). With respect to this possible mechanism, it is interesting that donor 1 did not possess a high-responding gene (i.e., Bw52). The failure of A2-associated responses in other donors who lack Bw52 may not necessarily contradict this since it is possible that one of the other three HLA-A or -B locus genes might exert a similar effect on the response associated with the antigen. However, this type of control has not been confirmed in the CTL response to virus in humans (1).

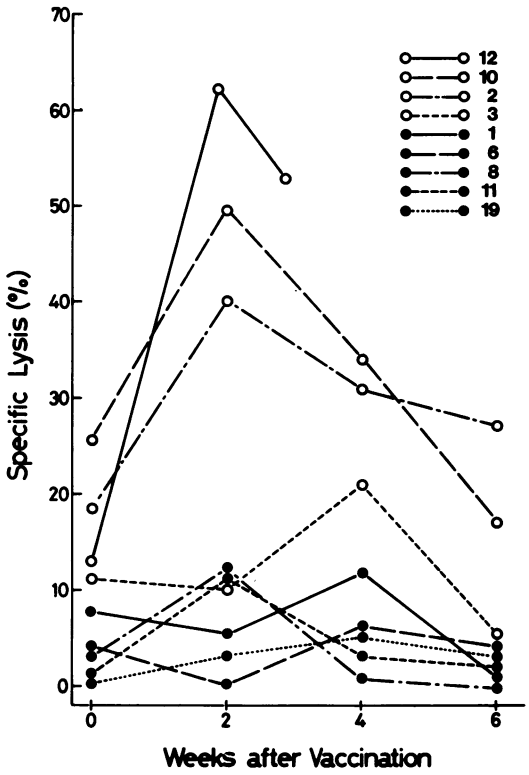

FIG. 2. Development of secondary cytotoxic response to mumps virus-infected autologous cells in four subjects who possess HLA-Bw52 (○) and in five subjects without the antigen (●) after booster immunization with live mumps vaccine. A target cell/effector cell ratio of 1:40 was used uniformly. The HLA type of each donor is given in the legend to Fig. 1, except for donor 19 (A9, –, B40, –).

Although the present results are still preliminary to the disclosure of all of the CTL response mechanisms, the above observations indicate the existence of the exquisite HLA-linked genetic control for the development of T cell-mediated immunity to mumps. From a clinical point of

view, it may be important to explore further whether such a genetically determined immune response is involved in the expression of various clinical manifestations of mumps. Such a study would also be helpful for the better understanding of the roles of the CTL response in the pathogenesis of the disease.

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