PARTICIPATION OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN ANTIBODY RECOGNITION OF VIRAL ANTIGENS EXPRESSED ON INFECTED CELLS*

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Recent analyses of immunologic specificity have demonstrated that, to a considerable extent, antigenic recognition involves not only the recognition of extrinsic antigenic determinants per se, but also the recognition of determinants contributed by antigen-presenting cells. For example, antigen-specific T helper cells are restricted in collaboration to cells syngeneic with the antigen-presenting cell responsible for their stimulation (1–5). Similarly, antigen-specific cytolytic T lymphocytes are restricted in effector function by the major histocompatibility complex (MHC)[1] of the stimulatory antigen-bearing cells (6–9). Such recognition, which apparently dominates the T cell specificity repertoire, has recently been implicated in antigen recognition by B cells as well (10–15). However, because many antibodies can bind to antigens in the absence of presenting cells, the portion of antibody binding that may be dependent on antigen presentation in the context of a presenting cell may generally be obscured by antibodies in the population capable of binding the antigen per se.

The present studies were carried out to determine at the monoclonal level the degree to which the antibody repertoire may also be restricted to the recognition of antigens in the context of a presenting cell. We have used syngeneic cell lines infected with the PR8 influenza strain (PR8) to stimulate individual primary B cells in the splenic focus system (16). This has permitted us to analyze the murine B cell repertoire specific for PR8 antigens as they are expressed on the cell surface and also to determine the extent to which the B cell repertoire may recognize PR8 determinants in the context of the immunizing syngeneic infected cell line. We have previously reported that the response to such a complex antigen is comprised of (*a*) monoclonal antibodies that recognize influenza determinants that are expressed on the isolated virion as well as on the cell surface of infected cells and (*b*) monoclonal antibodies that recognize the influenza determinants only when they are presented in the context of the infected cell (16). In this report, we explored the latter recognition in terms of the specific viral and cellular determinants that are necessary for the binding of these monoclonal antibodies. These analyses have taken advantage of the availability of both viral variants and H-2 mutants to define the specificity of such antibodies with apparent dual specificity. The findings indicate that a large proportion of syngeneic monoclonal antibodies specifically recognize determinants of the PR8 hemagglutinin (HA) as well

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[1] *Abbreviations used in this paper:* DMEM, Dulbecco's modified Eagle's medium; HA, hemagglutinin; MHC, major histocompatibility complex; NA, neuraminidase; PBS, phosphate-buffered saline.

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as H-2 antigenic determinants expressed on the cell surface and that the binding of these antibodies requires the simultaneous expression of both of these antigens.

Materials and Methods

Mice. Adult C3H/HeJ, BALB.K, C3H.SW, and C57BL/10 mice were obtained from the mouse breeding colony at Scripps Clinic and Research Foundation. Individuals 6–8 wk of age were used in the splenic fragment cultures. Neonatal mice 10–15 d old used in preparation of 1° kidney cell cultures were also obtained from the mouse breeding colony at Scripps Clinic and Research Foundation. These included BALB/c, C57BL/10, BALB.B, C3H.OH, B10.A(4R), B10.A(5R), D2.GD, and the following H-2K^b mutant strains: B6-C-H-2^{bm1}, B6-C-H-2^{bm4}, B6-C-H-2^{bm8}, B6-C-H-2^{bm9}, B6-C-H-2^{bm10}, and B6.C-H-2^{bm11}.

Viruses. Influenza virus PR8(A/PR/8/34[H1N1]) was obtained in the form of infectious allantoic fluid from Flow Laboratories, Inc., Rockville, Md. The following influenza A virus strains were obtained from Dr. Walter Gerhard, Wistar Institute, Philadelphia, Pa.: WSE(A/WSE/33[H1N2]); MEL(A/MEL/35[H1N1]); BEL(A/BEL/42[H1N1]); BH(A/BH/35/[H1N1]); WEISS(A/WEISS/43[H1N1]); CAM(A/CAM/46[H1N1]); and the recombinant virus Eq-PR8(A/Equine/Miami/1/63[Heq-2]-A/PR8/34[N1]). All virues were grown in the allantoic cavity of 10–11-d embryonated eggs. Upon isolation, the allantoic fluid was titered for hemagglutination activity and stored in 1-ml aliquots at −70°C until use. Virus was purified by adsorption to and elution from chicken erythrocytes followed by banding in a 15–45% sucrose gradient in an SW27 rotor by centrifugation at 18,000 rpm for 25 min (17). Purified virus was used in the radioimmunoassay as described previously (16, 18, 19).

Spleen Fragment Cultures. Spleen fragment cultures were carried out as described previously (16) for the stimulation of donor spleen cells with PR8-infected tumor cells. In both H-2^b and H-2^k haplotype mice, antigen-primed recipients syngeneic to the donor cells at H-2 and allogeneic at the heavy chain allotype locus were used to maximize T cell help while minimizing antibody-specific suppression of totally syngeneic responses (19). Antigenic stimulation was accomplished by intravenous injection of 2×10^6 PR8-infected L929 (H-2^k) cells for BALB.K (H-2^k) donor cells and 2×10^6 PR8-infected EL-4 (H-2^b) cells for C57BL/10 (H-2^b) donor cells. C3H/HeJ (H-2^k) mice and C3H.SW (H-2^b) mice were used as recipients for BALB.K donor spleen cells and C57BL/10 donor spleen cells, respectively. In both cases, recipients were primed with 1×10^7 PR8-infected cells of the appropriate type in alum and used 3–4 wk after priming.

Cell Cultures. BALB 3T3 clone 31 fibroblasts were obtained from the Cell Center at Salk Institute, La Jolla, Calif. L929 cells were obtained from Dr. Rolf Zinkernagel, previously from Scripps Clinic and Research Foundation. These cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% newborn calf serum (Irvine Scientific, Irvine, Calif.) and 50 μg/ml gentamicin (Scherring Corp., Kenilworth, N. J.). When infected with virus, the cells were removed from the monolayer with 0.25% trypsin and 0.024M EDTA in phosphate-buffered saline, pH 7.2 (PBS). 50 hemagglutination units of infectious virus in allantoic fluid were added to 2×10^6 cells in serum-free DMEM and incubated at 37° on 10% CO_2 for 4 h. The cells were then centrifuged, washed two times in DMEM with 10% newborn calf serum, and used for the preparation of assay plates as described below.

Primary Cell Culture. Kidneys to be used for the preparation of primary cell cultures were excised aseptically from 10–15-d neonates and placed in sterile PBS. They were then teased apart with forceps and placed in PBS containing 0.25% trypsin and 0.024M EDTA. The kidney tissue was stirred gently at room temperature for 10 min, then the supernate was decanted. Additional trypsin-EDTA was added, and the fragments were stirred gently at 4°C overnight. The cell suspension was then centrifuged at 12,000 rpm for 8 min at 4°C. The cells were resuspended in DMEM with 10% fetal calf serum and placed in two T-75 flasks for each neonate used. The cells were incubated at 37°C in 10% CO_2 for 3 d and then used for the preparation of cell assay plates as described below.

Preparation of Cellular Immunoadsorbents. Cell assay plates were prepared as described previously (16) except that poly-L-lysine (18,000 to 100,000 mol wt; Sigma Chemical Company, St. Louis, Mo.) was used in coating wells of polyvinyl plates in place of fetal bovine serum. Plates were incubated at room temperature for 1–2 h with 0.1 ml of poly-L-lysine (10 mg/100 ml of PBS) per well. The plates were then incubated with cells and fixed as previously described (16).

Radioimmunoassay. 25 μl of culture supernatants collected between days 9 and 21 of fragment culture were assayed for the presence of antibody through the use of a solid-phase radioimmunoassay, as previously described (16, 18–20). Pooled supernatants of each positive culture were assessed for specific reactivity using a panel of viruses or virus-infected cells. According to previously established criteria (20), reactivity to any given target was judged negative if binding was <10% of that to the homologous target. Positive reactivity was generally ≥80% of the binding of the monoclonal antibody to the homologous target.

Results

The Frequency of Monoclonal Responses Restricted to PR8-infected Cells. In a previous report (16), we presented the experimental procedures required to maximize in vitro monoclonal responses by B cells derived from nonimmune BALB.K mice (H-2^k) stimulated with syngeneic PR8-infected tumor cells. It was demonstrated that approximately one-third of the monoclonal antibodies were capable of binding determinants on the isolated virion and that these antibodies were equivalent in frequency and diversity to those derived by stimulation with the isolated virion per se in terms of the recognition of available HA and neuraminidase (NA) determinants. 58% of the monoclonal antibodies bound only to PR8-infected tumor cells and not to the isolated virion. To determine whether such recognition was a novel property related in some way to the transformed state of the cell used in screening, the antibodies were next analyzed for their capacity to bind PR8-infected primary kidney fibroblasts derived from BALB.K mice. 94% of the antibodies that bound PR8 in the context of L929 cells were also capable of binding PR8 in the context of BALB.K kidney fibroblasts. Because primary kidney cells, unlike L929 cells, are capable of supporting a productive influenza infection (21), the reactivity of these antibodies with infected BALB.K kidney fibroblasts demonstrates that the vast majority of antibodies recognizing only infected cells are not recognizing PR8 determinants unique to the L929 tumor cell, nor determinants expressed only on nonproductively infected cells.

A similar analysis was carried out with nonimmune C57BL/10 B cells (H-2^b) responsive to syngeneic PR8-infected EL-4 cells. The overall frequency of the response as assayed on PR8-infected BALB.B cells was 1 in 40,000 B cells. 66% of these antibodies bound PR8 in the context of infected BALB.B cells and not isolated PR8 virion.

To dissect the fine specificity of those monoclonal antibodies that required for their binding the expression of both viral and cell surface antigens, we included for further analysis only those antibodies that demonstrated reactivity with PR8-infected syngeneic kidney fibroblasts but not with either uninfected cells or the purified virion. In addition, culture fluids that demonstrated partial reactivity (>10% but <80% of the binding to the homologous PR8-infected cell line) to any one of the tested infected cell targets were considered ambiguous in their specificity and were excluded from further consideration. The use of these criteria arbitrarily excluded from further analysis all antibodies whose binding may have been influenced by other antigens indigenous to the tumor cell lines or antigens peculiar to a nonproductive infection. In spite of the exclusion of all antibodies with potentially ambiguous binding specificity, the monoclonal antibodies that fulfilled the above criteria and were thus included in the analyses below represented the products of a majority of nonimmune BALB.K or C57BL/10 spleen cells responsive to PR8-infected L929 or EL-4 cells, respectively.

The Fine Specificity for Viral Determinants of PR8-infected Cell-specific Monoclonal Antibodies. To determine which viral antigens were recognized by antibodies specific for PR8-infected H-2^k cells 112 monoclonal antibodies were analyzed for their capacity to bind L929 cells infected with equine PR8, which differs in HA but shares NA and other viral proteins with PR8. 19% of the antibodies that bound PR8-infected L929 cells were capable of binding equine PR8-infected L929 cells; thus, 81% of the antibodies apparently recognized the HA moiety. Table I presents an analysis of the specificity for HA of 34 monoclonal antibodies using a panel of L929 cells infected with one of six H1 influenza strains that are antigenically cross-reactive with PR8. It can be seen that over one-half of these antibodies recognized only PR8 and none of the other six strains. Additionally, most of those antibodies that bound to other H1 HA in the context of L929 cells bound to only one or two additional viruses. These findings contrast with previously reported reactivity patterns obtained for monoclonal antibodies that recognize determinants on the purified virion generated by stimulation with either the purified PR8 virion or with PR8-infected L929 cells, wherein many monoclonal antibodies recognized HA determinants common to several H1 strains (16, 18).

In an effort to conserve antibodies for analyses of H-2 recognition, only a small survey was carried out with monoclonal antibodies derived from C57BL/10 B cells that recognized PR8-infected BALB.B kidney fibroblasts. Of 14 antibodies analyzed, 12 demonstrated differential recognition amongst BALB.B kidney fibroblasts infected with the various H1 influenza strains (data not shown). Thus, the vast majority of monoclonal antibodies specific for PR8-infected H-2^b cells recognized determinants on the PR8-HA molecule.

The Fine Specificity for H-2 Antigenic Determinants of PR8-infected Cell-specific Monoclonal Antibodies. The above results clearly demonstrate that a substantial proportion of monoclonal antibodies can be highly virus-specific and yet their recognition is dependent on cellular presentation. Whereas it is possible that such antibodies

TABLE I

*Reactivity Pattern Analysis of PR8 (H1N1)-infected Cell-specific Monoclonal Antibodies against Cells Infected with Heterologous H1 Viral Strains**

			BH	+	+	+	−	+	−	−	−
			WSE	+	+	−	+	−	+	−	−
			MEL	+	−	+	+	−	−	+	−
WEISS	CAM	BEL									
+	+	+		—	—	—	3‡	—	—	—	—
−	+	+		—	—	—	—	—	—	—	3
+	−	+		—	—	—	—	—	—	—	3
+	+	−		—	—	—	—	—	—	—	3
−	−	+		—	—	—	—	—	—	—	21
−	+	−		—	—	—	—	—	—	—	3
+	−	−		—	—	—	3	—	3	—	6
−	−	−		—	—	—	—	—	—	—	52

* The monoclonal antibodies in this analysis were obtained from BALB.K donor spleen cells stimulated with PR8-L929 cells and include only those that recognized both PR8-infected L929 cells and PR8-infected BALB.K primary kidney cells but not purified virus or uninfected cells form both sources.

‡ Results are expressed as the percentage of 34 monoclonal antibodies showing a particular reactivity pattern.

recognize complex determinants that include both viral and cell surface antigenic contributions, it is also possible that such recognition reveals specificities that are unique to the infected state, such as viral-encoded determinants that are expressed only on the surface of infected cells and are not present or accessible in the intact virion. In an effort to determine the extent to which the recognition by monoclonal antibodies of PR8-infected cells could be attributed to the presence of cell surface antigens, particularly those encoded by the MHC, binding was assessed on PR8-infected kidney fibroblasts derived from MHC allogeneic strains.

Table II represents an analysis of 93 monoclonal antibodies derived from BALB.K B cells stimulated with PR8-infected L929 cells that bound PR8-infected BALB.K kidney fibroblasts but not the purified PR8 virion. These antibodies were assessed for their ability to bind PR8-infected 3T3 cells of BALB/c origin ($H\text{-}2^d$) and PR8-infected C57BL/6 kidney fibroblasts ($H\text{-}2^b$). 19% of the antibodies bound all three PR8-infected cell lines. It is not possible at this time to delineate from within this particular set those antibodies that may have recognized virus determinants in conjunction with H-2 determinants common to all three strains as opposed to those antibodies that may have recognized viral antigens that are unique for infected cells or expressed in conjunction with non-H-2 cell surface antigens.

A total of 21% of the antibodies recognized PR8-infected BALB.K cells as well as only one of the other two infected cell lines. As a result of such discriminate recognition, it is less likely that these antibodies recognized viral determinants unique to infected cells, and it is more likely that these antibodies recognized PR8 determinants in conjunction with determinants expressed on H-2-encoded molecules. If this is so, it would imply that antibody recognition of viral antigenic determinants includes recognition of H-2 determinants shared by different haplotypes. Finally, the majority of antibodies (60%) were restricted to recognition of only the H-2 syngeneic-infected cell line. Whereas the limited extent of the panel used in these studies does not permit an unambiguous attribution of these latter specificities, such complex recognition of PR8 determinants and determinants unique to $H\text{-}2^k$ antigens would be most consistent with the H-2-restricted recognition generally associated with T cell specificity (9).

To further define the precise recognition of these antibodies, 24 antibodies that recognized PR8-BALB.K-infected cells and not cells of the two different haplotypes were analyzed for their capacity to bind PR8-infected kidney fibroblasts derived from two strains expressing either the K end or D end of the $H\text{-}2^k$ haplotype. The reactivities of these antibodies are shown in Table III. One of these antibodies recognized both PR8-infected C3H.OH (K^d,D^k) and PR8-infected B10.A(4R) (K^k,D^b) kidney cells and therefore, may have recognized either a determinant shared by both

TABLE II

*MHC Restriction of Infected Cell-specific Monoclonal Antibodies**

PR8-BALB.K ($H\text{-}2^k$)	PR8-3T3 ($H\text{-}2^d$)	PR8-B6 ($H\text{-}2^b$)	Number	Percent
+	−	−	56	60
+	+	−	10	11
+	−	+	9	10
+	+	+	18	19

* The monoclonal antibodies in this analysis were obtained from BALB.K donor spleen cells stimulated with PR8-infected L929 cells and include only those that recognized both infected L929 cells and infected BALB.K cells but not purified virus or uninfected cells.

TABLE III

*H-2 Gene Products Recognized by PR8-H-2^k-specific Monoclonal Antibodies**

PR8-C3H.OH (K^d,D^k)	PR8-B10.A(4R) (K^k,D^b)	Number of antibodies	Percent of total
+	−	18	81
−	+	3	14
+	+	1	5

* The monoclonal antibodies in this analysis were obtained from BALB.K donor spleen cells stimulated with PR8-L929 cells and include only those that recognized PR8-infected BALB.K cells but not PR8-infected 3T3 or C57BL/6 cells (from Table II).

TABLE IV

*H-2 Gene Products Recognized by PR8-H-2^b-specific Monoclonal Antibodies**

PR8-BALB.B (K^b,D^b)	PR8-B10.A(5R) (K^b,D^d)	PR8-D2.GD (K^d,D^b)	Number of antibodies	Percent of total
+	+	−	40	73
+	−	+	0	0
+	+	+	15	27

* The monoclonal antibodies in this analysis were obtained from C57BL/10 donor spleen cells stimulated with PR8-infected EL4 cells and include only those that recognized PR8-infected BALB.B cells but not purified virus or uninfected cells.

TABLE V

*Reactivity Pattern Analysis of PR-8-infected H-2K^b Cell-specific Monoclonal Antibodies against PR8-infected H-2K^b Mutant Kidney Fibroblasts**

			bm9	+	+	+	+	−	−	−	−
			bm10	+	+	−	−	+	+	−	−
			bm11	+	−	−	+	+	−	+	−
bm1	bm4	bm8									
+	+	+		6‡	—	—	—	—	—	—	—
+	+	−		—	3	3	—	—	—	—	—
+	−	−		—	—	3	—	—	—	—	—
+	−	+		—	3	—	—	—	—	—	—
−	+	+		—	—	—	—	—	—	—	—
−	+	−		—	—	—	—	—	—	—	—
−	−	+		24	—	—	—	16	6	—	—
−	−	−		18	3	3	3	—	3	—	6

* The monoclonal antibodies in this analysis were obtained from C57BL/10 donor spleen cells stimulated with PR8-EL4 cells and include only those that recognized PR8-infected B10.A(5R) cells but not PR8-infected D2.DG cells (from Table IV).

‡ Results are expressed as percentage of 36 monoclonal antibodies showing a particular reactivity pattern.

the K end and D end of H-2^k or, alternatively, a non-H-2 determinant expressed neither on the PR8-infected 3T3 nor B6 cells. Three antibodies (12%) recognized PR8-infected B10.A(4R) cells but not PR8-infected C3H.OH cells, indicating that the specificity of these antibodies may be ascribed to the recognition of the H-2K^k-encoded determinants in conjunction with PR8 determinants. The vast majority of monoclonal antibodies (81%) recognized PR8 only in the context of cells syngeneic with the infecting cell line in the D end of the H-2^k locus, which is distinct from CTL

recognition of influenza-infected H-2^k cells, wherein the restriction appears to favor K end recognition (9).

To examine the generality of these findings and also to enable an unambiguous delineation of the H-2 contribution to determinant recognition, a similar analysis was carried out using monoclonal antibodies derived from C57B/10 B cells that had responded to PR8-infected EL-4 cells (H-2^b). As mentioned above, >60% of the antibodies derived from this stimulation demonstrated unique recognition of virus in conjunction with infected cells. 55 monoclonal antibodies that recognized PR8 determinants in conjunction with BALB.B-infected kidney fibroblasts were analyzed for their capacity to bind PR8-infected B10.A(5R) (K^b,D^d) and PR8-infected D2.GD (K^d,D^b) kidney fibroblasts. In Table IV it can be seen that 15 of these antibodies bound to both cell lines. Because these antibodies were not prescreened for dependence of their recognition on the expression of H-2^b per se, the specificity of these antibodies, like some of those in Table II, could not be attributed to the expression of a particular cell surface antigen. Surprisingly, the remaining 40 antibodies appeared to require the presence of the H-$2K^b$ molecule to effect recognition. Such specificity differs from CTL recognition of influenza-infected H-2^b cells where the predominant recognition is apparently for the D end of the H-2^b haplotype (9).

To demonstrate the participation of the H-$2K^b$ molecule per se in the recognition of these monoclonal antibodies, an analysis was carried out with 36 of the monoclonal antibodies whose specificity depended on the presence of both PR8 and H-$2K^b$, using a panel comprised of PR8-infected kidney fibroblasts derived from six different H-$2K^b$ mutant strains. These mutants differ from the strain of origin by only one or two amino acid substitutions in the K^b molecule (22). The results of this analysis are presented in Table V. It can be seen that only two of these antibodies (6%) recognized not only H-$2K^b$ but all of the mutants as well. The remaining 34 antibodies bound to PR8-infected H-$2K^b$ cells but did not bind to one or more of the panel of infected K^b mutants. Thus, even antibodies (included in this analysis) that delineate quite closely related HA structures can, at the same time, discriminate H-2-encoded molecules at the level of single amino acid replacements. Additionally, it appears that these antibodies recognize a heterogeneous set of restricting elements in their recognition of H-$2K^b$ determinants.

Discussion

The finding that immunologically competent cells can be restricted to the recognition of a variety of antigenic determinants only in the context of cells expressing syngeneic H-2-encoded antigens has generated considerable interest in the mechanistic basis for complex antigenic recognition (2, 6–8, 23–26). Although such complex recognition is characteristic of T cell specificity, there have been reports that imunoglobulins can also be restricted to the recognition of certain antigenic determinants in the context of cell surface antigens (10, 11). Similarly, although many studies have indicated that B cell stimulation need not be haplotype restricted (4, 27–29), a few recent investigations have implied that under certain conditions B cells may only be stimulated by antigen presented in the context of cells of an appropriate H-2 haplotype (12–15).

Because the antibody combining site for antigen has been carefully defined at the three dimensional structural level (30, 31), antibodies that recognize a defined antigen

in conjunction with determinants of cell surface antigens encoded by the H-2 locus could serve as excellent models for defining potential mechanisms of complex antigen recognition. To maximize the chances of observing antibodies exhibiting complex recognition, we analyzed B cell responses at the monoclonal level against influenza infected cell lines, an antigenic system for which complex recognition by cytolytic T lymphocytes has been carefully established (9, 20, 32). It was reasoned that at the monoclonal level antibodies recognizing viral determinants per se would not obscure the detection of antibodies whose binding required both viral antigenic determinants and antigenic determinants contributed by cell surface antigens. In a previous publication (16), we demonstrated the general parameters for obtaining this response and verified the efficacy of the stimulatory regimen on the basis that the component of the monoclonal response to influenza-infected cells that also recognized influenza determinants on the purified virion was comparable in frequency and diversity to responses to the same determinants when the purified virion was used for stimulation. The findings revealed that superimposed on the monoclonal response of B cells recognizing virion determinants per se was an even more vigorous response that recognized influenza determinants uniquely presented on the cell surface.

The findings in this report demonstrate that the majority of monoclonal antibodies uniquely specific for PR8-infected cells are dependent for their binding on the simultaneous presence of viral determinants and determinants of cell surface antigens encoded within the H-2 locus. It is important to point out that this recognition phenotype is not a rare or fortuitously occurring specificity. For example, in C57BL/10 mice approximately 1 in 60,000 splenic B cells of conventional nonimmune mice recognizes HA determinants present only on infected cells, whereas 1 in 150,000 B cells recognizes HA determinants expressed on the virion. In view of these results, it is possible that at least some antibodies that appear to recognize isolated virion determinants may also recognize complex determinants, including cell surface antigens, but recognize virion determinants with a sufficient affinity in the absence of cell surface antigens to be detected by radioimmunoassay using the virion alone.

The use of the influenza antigen in this study was predicated in part on the availability of a set of closely related viruses characterized by small differences within the HA moiety. Because monoclonal antibodies discriminate determinants shared to a greater or lesser extent amongst these variants, it has been possible to obtain a high degree of repertoire resolution of monoclonal antibodies recognizing the PR8-HA on the basis of reactivity pattern analysis (16, 18). Such an analysis carried out on the monoclonal antibodies that required both determinants of the influenza hemagglutinin and cell surface antigens have proven quite revealing in two ways. First, the fact that these antibodies discriminate amongst cells infected with influenza strains differing primarily in HA affixes the specificity, in terms of virus recognition by these antibodies, to the HA moiety. Second, the determinants recognized by antibodies exhibiting complex recognition appear to be predominantly "private" PR8-HA specificities or determinants shared by very few H1 strains. The finding that the HA determinants recognized by these antibodies is not a random representation of potential HA determinants may indicate considerably greater constraints with respect to the breadth of determinant recognition in antibodies that must simultaneously discriminate cell surface antigens as well as viral antigens. Studies (32) of the precise HA recognition by monoclonal T cells restricted to influenza plus syngeneic cell

recognition have not progressed sufficiently to reveal whether such specificity constraints are unique to B cells that recognize complex antigenic determinants.

It remains a possibility that some of the monoclonal antibodies that recognize influenza determinants only in the context of an infected cell are recognizing influenza components that are uniquely present on the cell surface and not available in the mature virion. This is particularly true of those monoclonal antibodies whose specificity could not be affixed to a given H-2 determinant by virtue of the panels of allogeneic or mutant H-2 strains used. Similarly, it is possible that within this set of antibodies are those that recognize influenza determinants in the context of non-H-2 cell surface antigens. In view of such potential recognitions, it is perhaps surprising that so great a majority of PR8-infected cell-specific monoclonal antibodies depend on the expression of H-2 determinants for their binding. Thus, it would appear that, similar to restrictions observed in the T cell repertoire, the B cell repertoire is significantly skewed towards recognition of complex antigens involving both viral and H-2-encoded determinants.

Also similar to T cells specific for influenza-infected syngeneic cells, antibodies show a clear preference for PR8 plus antigens encoded by only one end of a given H-2 haplotype. However, there is a striking disparity between the T and B cell repertoires of the H-2 haplotypes analyzed, in that the preference of BALB.K antibodies was for antigens encoded by the D end of the $H\text{-}2^k$ locus, and the preference of C57BL/10 antibodies was for the K end of the $H\text{-}2^b$ locus. Previous reports (9) for CTL specificity of influenza-infected $H\text{-}2^k$ and $H\text{-}2^b$ cells by syngeneic CTL have revealed the opposite preference in both instances. Although a sample of two haplotypes is an inadequate base for firm conclusions, if subsequent studies of other haplotypes and other antigens reveal similar disparities, such findings could reflect significant differences in the processes responsible for establishment and selection of the T vs. B cell repertoires. In considering the high proportion of infected cell-specific antibodies that are restricted by H-2 K or D end molecules, it is important to note that these findings do not address the possibility of I-region-restricted influenza-specific antibodies, as the stimulatory cells used in these studies were tumor cells that do not bear I-region antigens.

The most convincing evidence for the participation of determinants of H-2 antigens in the binding of antibody to PR8-infected cells comes from the analysis of the panel of PR8-infected $H\text{-}2K^b$ mutant kidney fibroblasts. Of the monoclonal antibodies that recognized PR8 only in the context of the K end antigens of the $H\text{-}2^b$ locus, the vast majority could be discriminated by their inability to bind certain PR8-infected $H\text{-}2K^b$ mutant cells. Other than the relatively low recognition of the infected bm1 line, which appears to share the fewest determinants with the native $H\text{-}2K^b$ molecule by monoclonal CTL analysis (33), little in the way of parallel reactivities between CTL recognition of the $H\text{-}2K^b$ mutants and the recognition of these mutants in conjunction with PR8 by monoclonal antibodies could be observed. Nonetheless, the finding that the vast majority of monoclonal antibodies could be discriminated by the mutant panel is consistent with the finding that some mutations suffice to totally restrict recognition of CTL for viral-infected syngeneic cells (34). Taken at face value, the finding that a significant proportion of monoclonal antibody molecules discriminates simultaneously both $H\text{-}2K^b$ mutants and viral HA variants represents strong evidence for recognition by antibodies of complex antigenic determinants as opposed to H-2 directed antigen processing.

The findings in this report introduce firm evidence that not only T cells but a substantial proportion of B cells may have their specificity determined by both viral and H-2 antigenic determinants. Because antibody molecules express singular combining sites, these findings demonstrate that complex recognition can be accomplished by a single combining site, thus obviating the necessity for proposing two independent and different receptors to account for complex antigen recognition. The demonstration of the relatively high frequency and relative ease of obtaining monoclonal responses to complex antigens that include determinants encoded by the MHC should provide fertile ground for future investigations of potential parallels in restriction and expression of the B and T cell repertoires.

Summary

The capacity of the antibody repertoire to recognize complex antigens on viral-infected cells was investigated at the level of monoclonal B cell responses. A majority of primary B cells responsive to PR8(H1N1)-infected H-2 syngeneic cells produced antibody that bound viral determinants only in the context of infected cells and not the isolated virion. An examination of the fine specificity of such antibodies revealed that most could be distinguished by a panel of cells infected with closely related heterologous H1 influenza strains. Indeed, most antibodies bound hemagglutinin determinants of PR8 exclusively, and few were broadly cross-reactive. An examination of the same antibodies for their recognition of cell surface antigens revealed that the majority recognized MHC-encoded antigenic determinants. Thus, most BALB.K ($H\text{-}2^k$) primary B cells responsive to PR8-L919 ($H\text{-}2^k$) cells produced monoclonal antibodies that bound PR8-antigens only in the context of $H\text{-}2D^k$-infected cells. Most C57BL/10 ($H\text{-}2^b$) B cells responsive to PR8-EL4 ($H\text{-}2^b$) cells produced monoclonal antibodies that bound PR8 antigens only in the context of $H\text{-}2K^b$-infected cells. These latter antibodies were further shown to recognize that $H\text{-}2K^b$ molecule by virtue of their capacity to be discriminated by a panel of PR8-infected $H\text{-}2K^b$ mutant cells. These findings demonstrate that much of the antibody repertoire is capable of highly specific complex recognition of viral antigenic determinants in the context of the appropriate MHC alloantigen.

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