Brief Definitive Report

MONOCLONAL ANTIBODIES AGAINST VIRAL DETERMINANTS ARE NOT RESTRICTED TO THE K/D END OF THE MAJOR HISTOCOMPATIBILITY COMPLEX

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It has been well established that T cells recognize foreign antigens in conjunction with products of the major histocompatibility complex (MHC) that are expressed on the surface of most cells (1). The question has been raised whether B cells and their products may recognize foreign antigen together with MHC antigens. Wylie et al. (2) have shown that influenza A virus–infected cells are recognized by monoclonal or oligoclonal antibodies in >50–70% of all examples only if the H-2 K/D region products are shared between the infected tumor cells or in vitro–cultured cells that were used for the induction of the B cell response and the infected cells that were used in the binding assay for antibody specificity. Comparable results have been obtained in a study (3) using simian virus 40 (SV40)-transformed tissue culture cell lines.

We investigated the specificity of monoclonal antibodies against the natural mouse pathogen lymphocytic choriomeningitis virus (LCMV). Monoclonal antibodies derived from 13 fusions of lymphocytes 6–13 d after initiation of a primary infection with myeloma cells were analyzed to evaluate whether MHC-restricted antiviral antibodies are frequent and therefore probably of biological relevance.

Materials and Methods

Mice, Virus, Immunization and Fusion. C57BL/6J (H-2^b) and C3H/HeJ (H-2^k) male and female mice were purchased from the Institut für Zuchthygiene, Tierspital, Universität Zürich. LCMV WE was originally obtained from Dr. F. Lehmann-Grube, Hamburg, Federal Republic of Germany, and LCMV Armstrong from Dr. M. B. A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, CA (1). The fusion of lymphoid cells with FO or Ag 8.653 cells was carried out according to standard procedures (4, 5).

Radioimmunoassay (RIA). A two-step RIA using ^{125}I-labelled anti–mouse Ig or three-step assays with either ^{125}I-labelled protein A or with biotinylated anti–mouse Ig and ^{125}I-labelled streptavidin were used to determine positive hybridoma supernatants. Briefly, virus-infected or -uninfected tissue culture cells were added at a concentration of 1–2 × 10^4 cells/well in 50 μl of RIA buffer PBS (1% BSA, 0.2% azide) to 50 μl of hybridoma supernatants. For hybridoma supernatants from group I (Table I), Mc57G (H-2^b) and D2 (H-2^d) cells were used for the screening and for group II supernatants, Mc57G and L929 (H-2^k) cells were used. After the addition of the cells to the supernatants, the preparations

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TABLE I

Summary of All Fusion Experiments Carried Out with Lymphoid Cells from LCMV-infected Mice

Group	Mouse strain	Virus strain	Infectious dose	Route of infection	Day of infection	Source of cells	Myeloma cell	Number of growing hybridomas
			PFU/mouse					
I	C57BL/6	WE	2×10^3	i.v.	−6	Spleen	FO	124
			2×10^4	i.v.	−5	Spleen	FO	17
			2×10^4	i.v.	−13	Spleen	FO	254
			2×10^5	i.v.	−13	Spleen	FO	230
			2×10^4	i.f.*	−4	IN‡	FO	6
			2×10^5	i.f.	−4	IN	Ag 8.653	171
			2×10^4	i.f.	−5	IN	Ag 8.653	21
			2×10^5	i.f.	−5	IN	FO	7
								Total: 830
II	C57BL/6	Armstrong	2×10^5	i.v.	−5	Spleen	FO	145
	C57BL/6				−10			231
	C3H				−5			49
	C3H				−10			74
	C57BL/6				−14 and −3§			2
	C57BL/6				−21 and −3			3
	C3H				−14 and −3			1
	C3H				−21 and −3			9
								Total: 514

* Mice were injected into the footpad.
‡ Inguinal lymph nodes were used for the fusions.
§ Mice received a booster injection 3 d before the fusion was carried out.

were incubated for 2 h at 4°C, and then washed three times before $3-5 \times 10^5$ cpm of ^{125}I-labelled compounds were added. After a further incubation of 2 h at 4°C, cells were washed repeatedly and radioactivity was determined. Binding of supernatants was considered to be positive if the absolute counts exceeded twice the background value, which was defined as the mean of 20% of the lowest values of all supernatants of the same plate. Those positive values always corresponded to >3–5 SD above the background value given by irrelevant monoclonal antibodies.

In all tests the following positive controls were included: monoclonal anti-LCMV IgG antibodies 6.2 and 24.A (kindly provided by Dr. M. Buchmeier, Scripps Clinic, La Jolla, CA [6, 7]) and monoclonal anti-K^b and anti-K^k antibodies.

Immunofluorescence. Mc57G, L929, and D2 cells were infected with LCMV and were either used without further treatment for determination of viral surface antigens or were grown on microscope-slides and then fixed in ice-cold acetone for detection of intracellular viral antigens. A 1:50 dilution of FITC-labelled goat anti-mouse Ig (Tago, Burlingame, CA) was used as second antibody. Thereafter cells were again washed repeatedly and scored for fluorescence under an inverted UV-light microscope. Two LCMV-specific monoclonal antibodies were used as a positive control for surface (6.2) (6) or internal viral antigens (24,A) (7). All hybridoma supernatants were also scored additionally on uninfected cells.

Results and Discussion

A total of 830 hybridomas were obtained after fusion of lymphoid cells from mice infected with LCMV for various times and were analyzed (Tables I and II). As indicated in Table II, all supernatants were first screened by cell RIA for reactivity on LCMV-infected Mc57G cells. The results show that 5–20% of all supernatants from growing hybridomas reacted with LCMV-infected cultured

TABLE II

Specificity of Supernatants Tested by RIA and Immunofluorescence

Group	Growing hybridomas	Positive superna- tants*	Virus-specificity on syngeneic cells‡		Virus-specificity on allogeneic cells§		Virus-specificity on fixed cells‖			Virus-specificity on living cells:¶ Mc57G and L929
							Mc57G	D2	L929	
I	124	15	Mc57G	2	D2	2**	0	0	0	0
	17	0	Mc57G	0		—	—	—	—	—
	254	27	Mc57G	4	D2	2	2	2	2	2
	230	24	Mc57G	3	D2	3	3	3	3	3
	6	2	Mc57G	1	D2	1	0	0	0	1
	171	16	Mc57G	4	D2	3**	0	0	0	1
	21	1	Mc57G	0		—	—	—	—	—
	7	0	Mc57G	—		—	—	—	—	—
	Total: 830	80		14						
II	145	29	Mc57G	1	L929	1				
	231	36	Mc57G	5	L929	4				
	49	3	L929	0		—				
	74	7	L929	1	Mc57G	1				
	2	0	Mc57G	—		—				
	3	2	Mc57G	0		—				
	1	0	L929	—		—				
	9	1	L929	0		—				
	Total: 514	78		7						

* Estimated as positive if absolute counts on infected cells exceeded twice the background value of control superantants (see Materials and Methods).

‡ Binding specificity tested on infected vs. uninfected Mc57G (H-2^b) cells.

§ Crossreactivity tested on infected D2 (H-2^a) or L929 (H-2^k) cells.

‖ Tested on acetone-fixed monolayer cells.

¶ Suspended cells were incubated with supernatants in the presence of 1% BSA and 0.2% azide at room temperature.

** All non-crossreactive supernatants subsequently showed loss of virus specificity.

cells. When these positive supernatants were further tested a high percentage was found that showed fluorescence on both infected and uninfected cells. These supernatants were excluded from the study and only the remaining supernatants that showed fluorescence exclusively on infected cells were regarded as specific for LCMV (Table II). These supernatants were however also reactive when tested on infected vs. uninfected allogeneic D2 (H-2^d; Table II, group I) or on the respective infected allogeneic target cells of group II. Essentially, the same results were obtained if hybridoma supernatants were tested in a cellular ELISA test (not shown). If supernatants were further tested for fluorescence on virus-infected acetone-fixed monolayer cells or on unfixed live cells, only 5 out of 11 supernatants positive by RIA on infected syn- and allogeneic cells showed specific reactivity. However, all of these supernatants showed positive reaction on all tested infected cells, irrespective of the H-2 haplotype (Table II, group I).

At least two questions are raised by our results that differ from the reports claiming that antibodies frequently bind to viral antigens only in association with the correct MHC determinant. First, are the differences in the analytical approach, both at the level of induction and of antibody detection, responsible for the data obtained and, second, what is the biological significance of the findings?

In contrast to the present study, other workers immunized mice with virus-infected tumor cell lines (2) or virus-transformed tissue culture cell lines (3) to induce T help for the induction of B cells in an adoptive transfer model. We could not find examples of MHC-restricted antibodies against LCMV when we

used live infected cells for the RIA or immunofluorescence studies. The discrepancies could be caused by the use of infected fixed cells for the RIA and the use of a relatively low technical cutoff point (>3 SD above background) for accepting RIA results as positive. This could have led to the detection of (weak) cell-surface determinants that are not really characteristic for the infecting virus and may be influenced by the fixation procedure (8). Furthermore, formal proof that the antibodies are really specific for virally induced cell surface antigens is lacking in both the influenza model (2) and the SV40 model (3), because antibodies that bind to purified virus and to infected cells were not further analyzed, since they are MHC-unrestricted. Similarly, SV40-transformed fibroblast cell lines may express many possible antigenic determinants and variants that may be related to SV40 transformation but characteristic for the individual cell clone rather than its particular H-2. The fact that in the SV40 study only one out of eight or nine antibodies shared the same specificity pattern may support this possibility.

Our results are in agreement with two reports that did not find any evidence for MHC-restricted antiviral antibodies after immunization of mice with syngeneic Sendai virus–infected spleen cells (9) or with influenza A virus–infected syngeneic EL-4 cells (10).

We therefore feel that the evidence that MHC-restricted virus-specific antibodies exist cannot be generalized, and that they may possibly be artifacts on technical grounds or rare examples. Our analysis of the antibody response during a primary or secondary infection with LCMV, a naturally occurring infectious disease in mice, suggests that antibodies specific for viral antigenic determinant are specific for this determinant independent of MHC determinants.

Summary

Monoclonal antibodies against lymphocytic choriomeningitis virus (LCMV), a natural, high-replicating, noncytolytic pathogen in mice, were obtained from fusions between myeloma cells and lymphoid cells of mice of different H-2 haplotypes at various times (4–24 d) after infection.

Supernatants from growing hybridomas were tested in a RIA, and ~15% of all supernatants were positive when tested for specificity on infected vs. uninfected cells of different haplotypes. Upon retesting for specific fluorescence, only some RIA^+ supernatants exhibited specific surface staining of acetone-fixed infected cells or unfixed infected cells. In all these experiments and using various detection methods we could not find antibodies with any preference of recognition of viral antigen in conjunction with the H-2 haplotype of the responder mouse.

The absence of H-2 restricted antibodies after a primary virus infection in vivo, whether assayed by RIA or surface immunofluorescence, suggests that antibodies obtained in other experiments (2, 3) using infected tumor cells for induction and in the RIA may not represent the general case.

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