to aggregate size, and therefore that secretion can be in some way inhibited, possibly, though not necessarily, by acrasin itself, acting extracellularly or intracellularly.

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Lysis mediated by T cells and restricted by H-2 antigen of target cells infected with vaccinia virus

VARIOUS virus infections lead to the formation of cytotoxic lymphocytes (CL), which are capable of killing virus-infected target cells[1–4]. Specific lysis of target cells infected with ^{51}Cr-labelled vaccinia virus could be observed when investigating the cell-mediated cytotoxic reaction to vaccinia virus[5]; the CL could be characterised as a T cell. The sensitised lymphocytes from C3H mice could only kill syngeneic L929 cells infected with vaccinia virus, whereas lysis by sensitised lymphocytes derived from DBA/2 mice was restricted to the syngeneic infected mastocytoma P815X2 cells. In the lymphocytic choriomeningitis infection the target cell lysis was shown to be restricted by H-2 antigen[6]. We report here experiments with primary fibroblasts of the mouse strains C3H, DBA/2 and the (C3H×DBA/2)F_1 generation were designed to affirm that the effector phase of virus-specific lysis of target cells mediated by T cells is restricted by H-2 antigen even in the vaccinia virus infection. Further experiments with H-2 alloantisera were performed to indicate the close local relationship between H-2 antigens and viral surface antigens.

Primary embryonic fibroblasts of the strains C3H (H-2^{kk}), DBA/2 (H-2^{dd}) and the (C3H×DBA/2)F_1 generation of the two strains (H-$2^{kd\ kd}$) were prepared, infected with vaccinia virus and used as targets for virus-specific cell mediated cytotoxicity. Lymphocytes were collected from mice sensitised 6 d previously. CL from the strains C3H (H-2^{kk}), DBA/2 (H-2^{dd}) and the F_1 (H-$2^{kd\ kd}$) generation were incubated with each type of the three infected target cells. The CL sensitised to vaccinia virus could lyse only syngeneic infected target cells or the semiallogenic infected cells from the F_1 generation, as was the case with permanent cell lines. Accordingly the hybrid CL from the F_1 generation mice were able to kill each of the infected targets (Table 1).

To investigate the local relationship between virus-induced surface antigens and H-2 antigens we tested whether H-2 alloantisera could inhibit the lysis of virus-infected target cells. These sera had no cross reactivity against vaccinia virus. Significant blocking of lysis of infected L929 fibroblasts or mastocytoma P815X2 was observed after incubation of antisera together with CL and infected target cells (Table 2). This effect was restricted by H-2; anti-H-2^{dd} sera could inhibit the virus-specific lysis of infected mastocytoma P815X2 cells, but not of infected L929 fibroblasts by CL from DBA/2 mice or C3H mice, respectively. Blocking could be observed up to a serum dilution of 1:256 when titrations of the H-2 antisera were performed (Fig. 1). Infected F_1 cells were used as targets and incubated with sensitised DBA/2 or C3H lymphocytes with addition of normal, anti-H-2^k or anti-H-2^d serum to determine whether the blocking was effective on the target cell or on the effector cell level (Table 3*a*). In this experiment the action of each CL was inhibited by both of the alloantisera, suggesting that the site of blocking is on the target cell. Secondly, CL from the F_1 generation were prepared and their activity was tested against the H-2^{kk} and H-2^{dd} target cells with the addition of H-2 alloantisera. The results clearly show that blocking was achieved only when an antibody was added, which was directed against H-2 antigens of the target cells (Table 3*b*). Thirdly, we preincubated either the target cell (L929 fibroblasts) or the CL (C3H) with the specific alloantiserum for 30 min at 37 °C, washed them twice with fresh medium and performed the test. As shown in Table 4 preincubation of CL was ineffective, whereas preincubation of the infected target cell caused inhibition of virus-specific lysis.

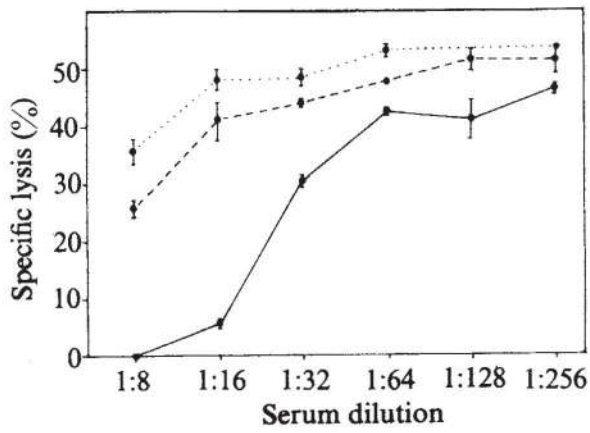

Fig. 1 Blocking effect of H-2 alloantisera on lysis of virus-infected target cells: L929 fibroblasts infected with vaccinia virus. Cytotoxic lymphocytes: spleen cells from C3H mice sensitised to vaccinia virus 6 d previously. Target cells and lymphocytes were incubated together with: ———, anti-H-2^{kk} serum; . . . . , anti-H-2^{dd} serum; - - - - , normal mouse serum.

The results support the concept that the action of virus-specific sensitised CL is restricted by the H-2 gene. Our findings in the vaccinia model are consistent with the observations in the LCM system[6] and similar results were obtained in the ectromelia virus system[4,13]. This restriction is clearly demonstrated for the effector phase *in vitro* and *in vivo*[7], but it may also be operative in the sensitisation phase[8]. Zinkernagel and Doherty[9] pointed out that this restriction does not reflect a physiological interaction with H-2 but rather the immunological surveillance against altered self components.

After addition of H-2 antisera to the *in vitro* test or preincubation of target cells with H-2 antisera significant blocking of target cell lysis can be achieved. This blocking effect is not caused by inhibition of the antigen-specific receptor of the cytotoxic T cell[10–12]. This observation indicates a very close topological relationship between the target antigens and H-2 on the cell surface. This target antigen

Table 1 Cytotoxic effect of CL on primary fibroblasts infected with vaccinia virus

Target cell*	Lymphocyte origin	% ^{51}Cr release†	% Specific lysis‡
C3H primary fibroblasts infected with vaccinia virus	Normal C3H	26.9±4.9	—
	Immune C3H	41.3±1.6	14.4
	Normal DBA/2	34.6±0.8	—
	Immune DBA/2	33.8±0.3	—
	Normal F_1 (C3H×DBA/2)	36.6±1.0	—
	Immune F_1 (C3H×DBA/2)	70.0±0.7	34.4
DBA/2 primary fibroblasts infected with vaccinia virus	Normal C3H	55.4±2.9	—
	Immune C3H	37.0±2.0	—
	Normal DBA/2	39.7±2.0	—
	Immune DBA/2	50.2±2.0	10.5
	Normal F_1 (C3H×DBA/2)	39.2±4.4	—
	Immune F_1 (C3H×DBA/2)	64.1±3.4	24.9
(C3H×DBA/2)F_1 primary fibroblasts infected with vaccinia virus	Normal C3H	51.1±1.3	—
	Immune C3H	62.6±2.1	11.5
	Normal DBA/2	46.5±1.1	—
	Immune DBA/2	59.8±1.8	13.3
	Normal F_1 (C3H×DBA/2)	30.4±1.2	—
	Immune F_1 (C3H×DBA/2)	58.8±2.3	28.4

*Primary embryonic fibroblasts from the strains C3H, DBA/2 and the (C3H×DBA/2)F_1 generation were prepared. The second or third passage of these cells was infected with 10 $TCID_{50}$ per cell of vaccinia virus (strain WR) 3 h before the cytotoxic assay. Production of virus-specific surface antigens was assayed by immunofluorescence. Virus-specific membrane fluorescence was visible after 2 h, reaching 80% stained cells after 20 h. CL were prepared[5] from spleens from mice infected intraperitoneally 6 d previously with 1 ml 10^6 $TCID_{50}$ ml^{-1} vaccinia virus. The test was performed using a ratio of 100 lymphocytes per target cell[5]. Incubation was performed over a period of 20 h. Spontaneous ^{51}Cr release from L929 or mastocytoma P815X2 target cells did not usually exceed 30%. Primary fibroblasts were much more sensitive to vaccinia virus infection and often showed high spontaneous release of the label; the addition of normal lymphocytes had a feeding effect and reduced spontaneous cytolysis.

†Each value represents mean±s.e.m. of $n=5$ wells, significance of specific lysis was affirmed by Students' t test ($P<0.01$).

‡Specific lysis was calculated by subtracting ^{51}Cr release in presence of normal lymphocytes from ^{51}Cr release in presence of sensitised cells. Spontaneous release of radioactivity from non-infected fibroblasts did not exceed 35%, CL had no unspecific activities on non-infected cells. Spontaneous release from infected fibroblasts was strain-dependent and varied between 30–50%.

may be either a specifically altered H-2 antigen (altered self), different from viral surface antigens, or it may be a non-specifically altered self plus viral surface antigen[9] and has an unknown topological relationship with both antigens. The blocking activity of H-2 antibodies favours both concepts. There can only be slight antigenic modifications otherwise the H-2 antiserum would no longer bind to the antigen. The blocking of the cytolytic reaction after addition of heterologous vaccinia antiserum[5] without cross reactivity to H-2 antigens seems to be more in favour of the second possibility. But it is also not at odds with the first assumption, as steric hindrance of the target antigen cannot be excluded. In preliminary experiments we have found different patterns of H-2 distribution on the surface of

Table 2 Effect of H-2 alloantisera on lysis of virus-infected target cells

Target cell	Lymphocytes	Serum	% ^{51}Cr release	% Specific* lysis	% Blocking
Mastocytoma cells infected with vaccinia virus	—	Normal	29.2±0.2	—	—
	Immune DBA/2	Normal	46.3±0.7	17.1	—
	—	Anti-H-2^{dd}	45.4±0.6	—	—
	Immune DBA/2	Anti-H-2^{dd}	46.5±1.6	0.9	16.2
	—	Anti-H-2^{kk}	36.0±1.0	—	—
	Immune DBA/2	Anti-H-2^{kk}	55.0±1.0	19.0	—
	Normal DBA/2	—	40.8±0.8	—	—
	Immune DBA/2	—	69.9±1.1	29.1	—
L929 cells infected with vaccinia virus	—	Normal	17.7±0.8	—	—
	Immune C3H	Normal	43.7±1.2	26.0	—
	—	Anti-H-2^{dd}	16.6±1.7	—	—
	Immune C3H	Anti-H-2^{dd}	52.3±2.1	25.7	—
	—	Anti-H-2^{kk}	18.2±1.0	—	—
	Immune C3H	Anti-H-2^{kk}	16.7±0.8	—	26.0
	Normal C3H	—	18.0±0.4	—	—
	Immune C3H	—	68.3±5.5	50.3	—

*Specific lysis was calculated by subtraction of lysis in presence of serum from lysis in presence of serum and lymphocytes.

†Blocking effect of H-2 alloantiserum was calculated by subtraction of lysis in presence of H-2 alloantiserum and lymphocytes from lysis in presence of normal serum and lymphocytes.

H-2 alloantisera were obtained from Dr J. G. Ray, Transplantation and Immunology Branch, NIAID, NIH, Bathesda, Maryland. We used as anti-H-2^{kk} serum D-3b, raised in the recipient–donor strains (C3H-H-2^{c}×129) anti-C3H; genotypes were (H-$2^{d/k}$×H-2^{bb}) anti-H-2^{kk}. This serum is cytotoxic for H-2 specificities 11, 23, 25, 52, and also haemagglutinating for specificity 3. As anti-H-2^{dd} serum we used D-31, raised in recipient–donor strains (B10×A) anti-B10.D2; genotypes were: (H-2^{bb}×H-$2^{k/d}$) anti-H-2^{dd}; anti-H-2 specificities were: 31, 34. Details of the raising and testing of these sera are described in the NIH catalogue[14].

Table 3 Site of blocking mediated by H-2 alloantisera

Target cell	Lymphocytes	Serum	% ^{51}Cr release	% Specific lysis	% Blocking
(*a*) (C3H × DNA/2)F_1 primary fibroblasts infected with vaccinia virus	—	Normal	47.0±3.0	—	—
	Immune DNA/2	Normal	59.8±3.4	12.8	—
	Immune C3H	Normal	60.4±1.9	13.4	—
	—	Anti-H-2^{dd}	43.2±1.7	—	—
	Immune DBA/2	Anti-H-2^{dd}	44.8±1.7	1.6	11.2
	Immune C3H	Anti-H-2^{dd}	44.2±1.0	1.6	12.4
	—	Anti-H-2^{kk}	50.2±1.9	—	—
	Immune DBA/2	Anti-H-2^{kk}	46.3±2.0	—	12.8
	Immune C3H	Anti-H-2^{kk}	41.1±1.5	—	13.4
F_1 primary fibroblasts infected with vaccinia virus	Normal DBA/2	—	46.8±0.9	—	—
	Immune DBA/2	—	59.9±1.8	13.3	—
Uninfected F_1 primary fibroblasts	Immune DBA/2	—	47.0±2.0	—	—
	Immune C3H	—	40.7±1.5	—	—
(*b*) L929 cells infected with vaccinia virus	—	Normal	26.6±1.0	—	—
	Immune (DBA/2 × C3H)F_1	Normal	67.6±2.6	40.9	—
	—	Anti-H-2^{kk}	36.9±3.1	—	—
	Immune (DBA/2 × C3H)F_1	Anti-H-2^{kk}	31.4±1.1	—	40.9
	—	Anti-H-2^{dd}	50.5±3.6	—	—
	Immune (DBA/2 × C3H)F_1	Anti-H-2^{dd}	67.6±2.0	41.0	—
	Normal (C3H × DBA/2)F_1	—	38.5±3.1	—	—
L929 cells infected with vaccinia virus	Immune (C3H × DBA/2)F_1	—	62.0±2.4	23.6	—
Uninfected L929 cells	Immune (C3H × DBA/2)F_1	—	18.9±1.8	—	—

Table 4 Effect of preincubating CL or targets with H-2 alloantisera on target lysis cell

Target cell	Lymphocytes	Serum	% ^{51}Cr release	% Specific lysis	% Blocking
L929 cells infected with vaccinia virus	—	Normal	17.9±0.8	—	—
	Immune C3H	Normal	33.8±2.4	15.9	—
	—	Anti-H-2^{kk}	18.6±1.9	—	—
	Immune C3H	Anti-H-2^{kk}	9.7±1.0	—	15.9
	Immune* C3H	Normal	29.4±0.6	11.6	—
	Immune* C3H	Anti-H-2^{kk}	13.1±1.3	—	11.6
	Immune† C3H	Normal	28.8±2.1	10.9	—
	Immune† C3H	Anti-H-2^{kk}	26.6±1.5	8.0	2.9
	Normal C3H	—	22.5±2.0	—	—
L929 cells infected with vaccinia virus	Immune C3H	—	39.4±2.0	16.9	—
Uninfected L929 cells	Immune C3H	—	19.9±1.2	—	—

*Target cells preincubated with serum.
†Lymphocytes preincubated with serum.

infected and non-infected cells by immunofluorescence. In absorption studies vaccinia-infected cells had a reduced capacity to absorb H-2 antisera. Secondly, preincubation of the infected cells with H-2 antisera reduced the vaccinia specific fluorescence. This again suggests a close topological relationship between both antigens.

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Measles virus receptor on human T lymphocytes

One of the factors which may determine whether or not a cell is susceptible to a particular virus is the presence in the cell membrane of a receptor or a binding site for the virus[1]. It has been demonstrated that certain viruses, including herpes and myxoviruses, can infect and multiply within lymphocytes[2,3]. Following von Pirquet's original observation[4] it is now well recognised that acute measles infection is associated with depression of cellular (T lymphocyte-dependent) immunity.