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Altered serological and cellular reactivity to H-2 antigens after target cell infection with vaccinia virus

MICE generate cytotoxic T lymphocytes (CTL) which are able to lyse virus infected target cells *in vitro* after infection with lymphocytic choriomeningitis virus (LCMV) and poxviruses[1–3]. CTL kill syngeneic and semiallogenic infected cells but not allogenic infected targets. Target cell lysis in these systems seems to be restricted by H-2 antigens, especially by the K or D end of the major histocompatibility complex (MHC). In experiments where virus specific sensitised lymphocytes kill virus infected allogenic target cells[4] the effector lymphocytes have not been characterised exactly. Recent investigations suggest that the active cell in this assay, at least in the measles infection, is a non-thymus derived cell (H. Kreth, personal communication). An H-2 restriction of cell mediated cytolysis (CMC) to trinitrophenol (TNP)-modified lymphocytes has also been described[5]. Zinkernagel and Doherty[6] postulated that the CTL is directed against syngeneic H-2 antigens and viral antigens and they suggested an alteration of H-2 induced by the LCMV infection. Earlier[7] we found a close topological relationship between H-2 antigens and the target antigen(s) responsible for CMC in the vaccinia system. Here we report experiments which were carried out to prove alteration of H-2 after infection of L-929 fibroblasts with vaccinia virus.

Alteration of H-2 antigens was studied by immunofluorescence. L-929 cells after 1 h infection with 10 $TCID_{50}$ per cell vaccinia virus strain WR and 19 h incubation in serum-free medium, as well as normal L-929 cells were stained by indirect immunofluorescence. L cells (5×10^6) were incubated in 100λ H-2^k (charge D 3b, raised in recipient-donor strains (C3H-H2° × 129) anti-C3H; genotypes ($K^dD^k \times K^bD^b$) anti-K^kD^k. This serum is cytotoxic for H-2 specificities 11, 23, 25, 52 and haemagglutinating for specificity 3, (ref. 8) for 30 min, 37 °C and 4 °C and after washing 3 times in PBS incubated in 100λ FITC-labelled rabbit anti-mouse IgG in the same conditions. After the cells were washed three times, viability was tested (> 90%) and slides were prepared without previous fixation of cells. All of the normal fibroblasts had a dense and regular fluorescence, whereas about 25% of the infected cells lacked H-2 fluorescence. The remaining 75% cells had an irregular and more coarse-grained staining.

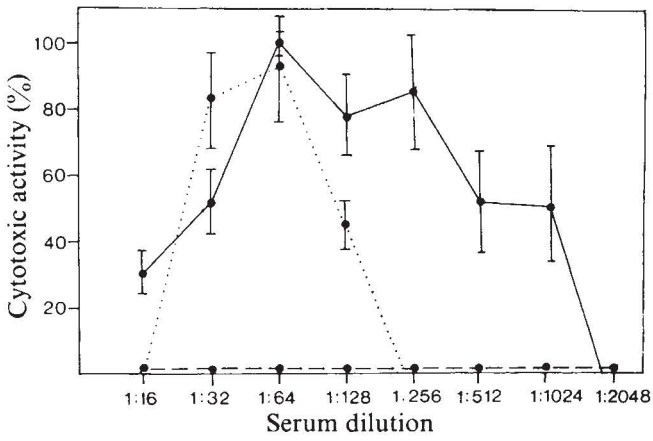

Fig. 1 H-2^k alloantiserum absorption with normal and vaccinia infected L-929 cells. ●———●, cytotoxic activity of anti H-2^k serum (^{51}Cr release) against C3H spleen lymphocytes. ●·····●, cytotoxic activity of anti H-2^k serum after 30 min absorption at 37 °C with 1×10^8 vaccinia virus infected L cells (20 h infected 10 $TCID_{50}$ per cell vaccinia strain WR). ●------●, cytotoxic activity of anti H-2^k serum after absorption with 1×10^8 normal L-929 cells. Serum dilutions were performed in Eagle's MEM. Each point represents $N = 4$ values ± s.d. Differences are statistically significant ($P < 0.001$). Maximal ^{51}Cr release by H-2^k alloantiserum before absorption was calculated as 100% cytotoxic activity (Antibody mediated ^{51}Cr release ranged from 40–65% of total incorporated chromium). Controls included spontaneous lysis of targets alone, lysis in presence of complement alone, H-2 antibodies alone, normal mouse serum alone and normal mouse serum together with complement.

Absorption studies were performed to quantify the possible H-2 alteration seen in immunofluorescence. Different numbers of viable infected and non infected L-cells (2.5×10^7–1×10^8) were incubated with 0.5 ml anti-H-2^k serum for 30 min at 37 °C. After this, the cytotoxic titre of the absorbed serum was tested in the ^{51}Cr-release assay using C3H spleen lymphocytes (H-2^k) as targets and guinea pig serum as complement source (Figs 1 and 2). Then the lysis of target cells was assayed by Trypan blue exclusion using rabbit complement. Both tests gave nearly the same results.

Serum D-3b was absorbed quantitatively five times, with comparable results. There was a constant reduction of H-2 alloantiserum absorbing capacity of L-929 cells infected with vaccinia virus (Fig. 1). Using 1.0×10^8 normal cells for absorption we found no cytolysis in the ^{51}Cr-release assay. The complete absorption was not simulated by anti-complementary activity of the H-2 serum since the absorbed serum showed no more H-2 fluorescence, and absorption with increasing amounts of normal L cells showed a stepwise decrease of cytolytic activity. Serum absorbed with the same amount of infected cells showed cytolytic activity up to a serum dilution of 1:128. After absorption with 2.5×10^7 and 5×10^7 cells the anti-H-2^k serum showed 20–40% more cytolytic activity when infected cells were used for absorption (Fig. 2).

Table 1 Blocking of CMC against target cells infected with vaccinia virus

Lymphocytes*	Target cell	Cr release†	Specific lysis‡
Normal DBA/2	L-929	20.1 ± 1.2	
Normal C3H	not infected	18.5 ± 0.7	
DBA/2 sensitised to H-2^k		64.4 ± 0.8	44.3§
C3H sensitised to vaccinia virus		19.4 ± 1.3	
Normal DBA/2	L-929	21.2 ± 0.9	
Normal C3H	vaccinia virus infected	23.4 ± 0.8	
DBA/2 sensitised to H-2^k		22.5 ± 0.7	
C3H sensitised to vaccinia virus		72.8 ± 1.4	49.4§

*Lymphocytes from a pool of six mice per group. Virus sensitisation was performed by intraperitoneal injection of 1 ml 1×10^6 $TCID_{50}$ ml^{-1} vaccina virus six days previously. Sensitisation to H-2^k was achieved by intraperitoneal injection of 1×10^8 C3H spleen lymphocytes 10 d previously. Killer–target cell ratio, 100:1.
†Mean of percentage ^{51}Cr release ± s.e.m. of 5 wells per group.
‡Specific lysis (%) = (^{51}Cr release (%) by sensitised lymphocytes) − (^{51}Cr release (%) by normal lymphocytes).
§Differences are statistically significant ($P < 0.001$).

The influence of target-cell infection on CMC was tested. Since the effector phase of the allograft reaction, which is reflected *in vitro* by the CMC of allogenic cells, is directed mainly against serologically defined (SD) antigens[10], modifications of these antigens should influence the degree of CMC. DBA/2 mice (genotype H-2^d) were sensitised to H-2^k by intraperitoneal injection of 1×10^8 spleen cells from C3H mice. Ten days later, the DBA/2 lymphocytes were collected and the cytolytic activity of the CTL tested against infected and non-infected L-929 target cells in a ^{51}Cr-release assay[3]. Table 1 shows the results of an experiment representative of the four carried out. After incubation of the CTL sensitised to H-2^k with the normal L cells we found a specific release of 44.3% of the incorporated chromium. There was, however, no specific lysis of the allogenic target cells infected corresponding to the serum absorption assays with vaccinia virus. In the immunofluorescence control the target cells had vaccinia virus surface antigen fluorescence and altered H-2 fluorescence as described above. In spite of serologically demonstrable H-2 antigens, the CTL could not kill the infected allogenic cells. With syngeneic attacker cells sensitised to vaccinia virus we observed significant specific lysis of the infected target cells.

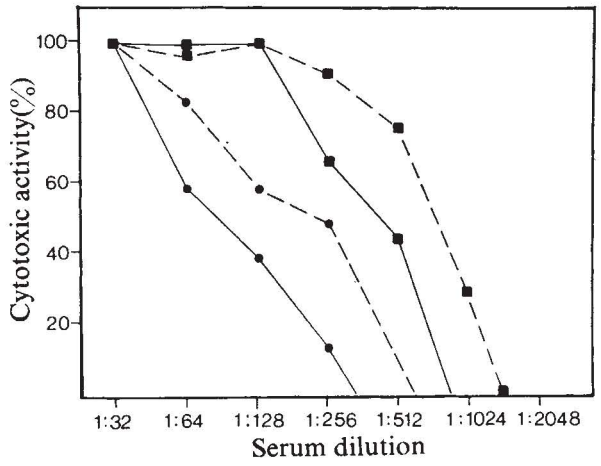

Fig. 2 Comparison of quantitative absorption by different numbers of vaccinia virus infected and normal L-929 cells. ●———●, H-2^k (0.5 ml) absorbed on 0.5×10^8 L cells; ●-------●, 0.5×10^8 vaccinia virus infected L cells; ■———■, 0.25×10^8 L cells; ■-------■ 0.25×10^8 vaccinia virus infected L cells. Maximal ^{51}Cr. release by H-2^k antiserum before absorption was calculated as 100% cytotoxic activity.

These data indicate that infection of L-929 fibroblasts with vaccinia virus may result in a reduction and an alteration of H-2 antigenic sites. It has been shown by serum absorption tests that tumour viruses can increase or diminish the level of H-2 specificities[11,12].

H-2 antigen expression is cell cycle dependent[13] and possibly the quantitative alteration after vaccinia virus infection could be caused by a virus-induced nonspecific blocking of the metabolism of membrane proteins. Using enteroviruses as infective agents we were not able to find a reduced H-2 alloanti-serum absorbing capacity or an inhibition of lysis of allogenically infected cells in the CMC. Expression of viral surface antigens seems, therefore, to be a prerequisite for antigenic alteration. Rearrangement of H-2 antigen specificities[14] accompanied by virus-induced inhibition of membrane fluidity does not seem to be a sufficient explanation for the inhibition of T-cell effector function, since antigenic recognition is possible after fixation of cells[15].

If the expression of H-2 in infected and non-infected cells were identical by serological means and infection induces only quantitative differences of H-2 antigen expression, the cells should by lysable in the CMC against alloantigens[16]. There is, however, not only slight reduction, but complete blocking of CMC. This could mean that there is a virus-induced qualitative change of H-2 antigenicity or a steric hindrance of the target antigen for the T cell sensitised to alloantigen. Different localisation of serologically and cellularly detectable antigenic sites on the same molecule could also explain the different results of antibody binding and allogenic CMC. The latter possibility is supported by the finding that CMC between cells identical in SD antigens and different only in H-2 alloantiserum absorbing capacity occurs[17–19]. Reduction of CMC against allogenic cells has been obtained by Gardner *et al.*[20] in the ectromelia system while modification of cells with TNP did not alter CMC to H-2 alloantigens[5]. These contradictory results may reflect the different sizes of the modifying agents or the different process and extent of alteration.

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Modulation of macrophage Fc receptor expression *in vitro* by insulin and cyclic nucleotides

The capacity of certain cells to bind antibody or immune complexes through receptors at the cell surface may be important in both inductive and effector limbs of the immune response. Macrophages[1], B lymphocytes[2], activated T lymphocytes[3] and certain tumour cells[4] express such receptors which interact with the Fc portion of IgG (Fc receptors). I have shown that guinea pig macrophages are heterogeneous in their capacity to bind antibody, that the expression of Fc receptors is greatly increased during cell activation *in vivo* and *in vitro* and that this activation of receptor expression is inhibited by a factor(s) present in fresh autologous serum[5]. Here I show that changes in macrophage Fc receptor expression *in vitro* are modulated by insulin and by cyclic nucleotides.

Normal peritoneal cells were extracted aseptically from untreated outbred guinea pigs by washing the peritoneal cavity with 25 ml of RMPI 1640 tissue culture medium supplemented with 10% foetal calf serum (FCS) and containing heparin (5 U ml^{-1}). Samples (1 ml) of this suspension, which contained approximately 1×10^6 macrophages per ml, were placed immediately in each chamber of dual tissue culture slides (Lab-Tek Products). Cells from more than one animal were never pooled. These cells were incubated at