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MODIFICATION OF H-2 ANTIGENIC SITES BY ENZYMATIC TREATMENT INFLUENCES VIRUS-SPECIFIC TARGET CELL LYSIS[1]

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Vaccinia virus-infected cells were treated enzymatically to remove H-2 antigenic sites. The effect of this procedure on virus-specific cell-mediated cytolysis (CMC) and virus-specific antibody-mediated cytolysis (AMC) was tested. Due to the inhibition of cellular proteinsynthesis by the vaccinia virus infection, H-2 antigenic sites were not resynthesized while there was a continuous production of viral surface antigens.

These cells with a high concentration of viral surface antigens and decreased H-2 determinants could be used as targets in the virus specific AMC. But they were not lysed in the virus specific CMC which emphasizes the significance of H-2 antigens during recognition of virus-specific determinants by T cells.

The action of cytotoxic lymphocytes (CL)[3] against target cells infected with certain viruses, such as lymphocytic choriomeningitis (LCM) virus, ectromelia virus or vaccinia virus, is H-2 antigen restricted (1-3). Specific lysis occurs only if attacker and target cells share serologically defined (SD) antigens of the major histocompatibility complex (MHC). In the vaccinia virus system, cell-mediated lysis can be inhibited by H-2 alloantisera binding to SD specificities of the target cell (4). Other experiments indicate that a virus infection causes a reduction of normal H-2 antigenic sites, probably by modifying them (2, 5). Such a virus-induced alteration was suggested earlier by Zinkernagel and Doherty in the LCM virus system (6). All these data indicate that a modified H-2 antigen, associated to the K or D end of the MHC, seems to be the target for virus-specific sensitized T cells. The same conclusion was drawn by Shearer *et al.*, who found a similar H-2 dependency of the T cell response by using trinitrophenyl (TNP) as modifying agent (7, 8).

In contrast to these results, Dennert reported lysis of TNP-modified syngeneic target cells by T cells sensitized against allogenic TNP-modified cells. His explanation was that CL not only act against the modified H-2, but also against the hapten (9). The blocking effect of viral antibodies on cell-mediated cytolysis (CMC) could be interpreted in the same way (3, 10).

Considering these conflicting results, we tested whether syngeneic H-2 is necessary for T cell action or if the agent modifying the cell surface, such as the viral surface antigen, suffices as a target for T cell-mediated lysis. Removal of H-2 antigenic sites from the target cell decreases cytolysis by allogeneic-sensitized CL (11). This inhibition is fully reversible after resynthesis of the H-2 antigens. Removal of neuraminic acid from the target cell surface increases CMC (12) and antibody-mediated cytolysis (AMC) without influencing H-2 alloantibody-absorbing capacity (13). In our experiments infected cells were treated with different enzymes in order to obtain target cells with decreased H-2 antigenic sites but without alterations of the concentration of viral surface antigens.

MATERIAL AND METHODS

Animals

C3H/TIF inbred mice, haplotype H-2^k (purchased from G. L. Bornholdgård, Ry, Denmark) aged between 6 and 10 weeks were used.

Virus

Vaccinia virus, strain WR, propagated on VERO cells (monkey kidney cells) was diluted to about 10^6 $TCID_{50}$/ml in Eagle's minimal essential medium (MEM, Behringwerke, Marburg/Lahn, W. Germany) supplemented with 100 μg/ml streptomycin and 100 IU/ml penicillin (1% SP).

Antisera

Anti-vaccinia serum. Anti-vaccinia serum was raised in rabbits by 3-fold challenge with 5 ml vaccinia virus given intramuscularly at 2-week intervals after intracutaneous sensitization. The animals were bled 1 week after the last injection. Sera were inactivated and stored at −70°C. The cytotoxic titer of the sera measured in a ^{51}Cr-release assay, using infected L-929 cells as targets and guinea pig serum as complement source, was about 1024 to 2048. An anticomplementary activity of the sera was demonstrable to a dilution of about 1/8.

Anti-H-2 serum. Anti-H-2 serum was obtained from D. J. G. Ray, Transplantation and Immunology Branch, NIAID, National Institutes of Health, Bethesda, Maryland. We used charge 3b, raised in recipient-donor strains (C3H-H-2° × 129) anti-C3H, genotypes (H-$2^{d/k}$ × H-2^b) anti-H-2^k. The serum is mainly directed against specificities coded by H-2 K genes (14). No cross-reactivity with other private H-2 specificities

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[3] Abbreviations used in this paper: CL, cytotoxic lymphocyte; AMC, antibody-mediated cytolysis; CMC, cell-mediated cytolysis; FCS, fetal calf serum; IF, immunofluorescence; LCM, lymphocytic choriomeningitis; MHC, major histocompatibility complex; SD-antigen, serologically defined antigen; VCN, *Vibrio comma* neuraminidase; TNP, trinitrophenyl.

could be observed by immunofluorescence or complement-dependent antibody-mediated cytolysis.

Target cells

L-929 cells (haplotype H-2^k), obtained from Dr. Lehmann-Grube, Hamburg, were cultivated in Eagle's MEM supplemented with 10% inactivated calf serum and 1% SP.

Infection of target cells. About 5×10^6 to 1×10^7 L-929 cells growing as monolayer cells in glass bottles were washed with fresh serum-free medium and then incubated with 10 to 15 ml vaccinia virus suspension on a rocker-platform at 37°C. After 2 hr the virus dilution was discarded and fresh serum-free medium was added. The cells were used for tests 18 to 24 hr later.

Enzymatic treatment

The following enzymes were used: 1) trypsin from bovine pancreas, crystallized, lyophilized, 2 units/mg (Merck, Darmstadt, W. Germany, lot 24579); 2) pronase E lyophilized, 70 PUK/mg (Merck, lot 2433); 3) papain, water soluble 3.5 M Anson U/mg (Merck, lot 7144); 4) neuraminidase from *Vibrio comma* (VCN), 500 neuraminidase units/ml (Behringwerke, Marburg/Lahn, W. Germany). The enzymes were dissolved and diluted in serum-free MEM. Used enzyme concentrations may be seen in Table I.

Cells prepared for enzymatic treatment were washed twice with serum-free medium and diluted to a concentration of 2.5×10^6 cells/ml. The enzymatic treatment was performed at 37°C with intermittent shaking at pH 6.5 to 7.0 for 20 min (proteolytic enzymes) or 30 min (VCN). Treatment with papain was carried out in the presence of 0.01 M cystein (L(+) Cysteine chloride, Merck, lot 2839). After enzymatic treatment the cells were washed three times with MEM supplemented with 20% fetal calf serum (FCS).

Cells were counted and viability was determined by the trypan blue exclusion test. Under the enzyme concentrations used viability did not fall below 70 to 80%. The cells were stored in Eagle's MEM supplemented with 20% FCS before use. For virus-specific cytotoxic assays, carried out 4 hr after enzymatic treatment, cells were labeled 1 hr before with about 100 μCi ^{51}Cr (sodium chromium; Amersham, Buchler, Braunschweig No. CJS1P, specific activity 100 to 200 mCi/mg Cr) per 5×10^6 cells. For allogenic CMC, cells were labeled before enzymatic treatment and used as targets immediately after treatment.

TABLE I

Schedule of enzymatic treatment[a]

Enzyme Concentration	Trypsin	Pronase	Papain	VCN
	μg/ml	*μg/ml*	*μg/ml*	*units/ml*
1	0.5	0.5	5	5
2	1	1	10	20
3	2	2	20	50
4	5	5	50	100
5	10	10	100	200
6	20	20	200	

[a] Enzymatic treatment: 2.5×10^6 overnight vaccinia virus-infected L-929 cells suspended in 1 ml MEM were incubated with different concentrations of enzymes at pH 6.5 to 7 for 15 to 20 min (proteolytic enzymes) or 30 min (VCN) at 37°C humidified atmosphere with 8% CO_2. Enzymatic action was stopped by addition of 2 ml FCS and 3-fold washing. Viability was tested with trypan blue; under the enzyme concentrations used viability did not fall below 80%. The cells were stored in Eagle's supplemented medium + 20% FCS at 37°C and labeled for 1 hr with ^{51}Cr before use. Controls were: enzymatically treated noninfected L-929 cells, untreated infected and noninfected cells, and cells treated with enzymes in the presence of Trasylol, a proteinase inhibitor (Trasylol, 5000 units, Bayer Leverkusen). Enzymatically treated cells were used as targets 4 hr later in virus-specific tests.

Immunofluorescence

5×10^6 infected or normal L-929 cells were incubated for 30 min 4°C with 0.1 ml anti-vaccinia serum from rabbit or anti-H-2 serum diluted 1/5 with medium. Cells were then washed three times with phosphate-buffered saline (PBS) and incubated for another 30 min with a fluorescein-isothiocyanate-conjugated anti-rabbit γ-globulin from goat or anti-mouse γ-globulin from rabbit (Behringwerke, Marburg/Lahn, W. Germany), at room temperature or at 4°C.

Antibody-mediated lysis

Vaccinia-specific AMC. 5×10^4 L-929 cells infected 24 hr previously were labeled 1 hr before with ^{51}Cr and then incubated in a volume of 0.05 ml in medium with 0.05 ml anti-vaccinia serum 1/10 diluted in microplates for 30 min at 37°C. Then 0.1 ml guinea pig complement was added. After 4 hr incubation at 37°C the cellfree supernatant and the cell pellet were separately removed, the radioactivity was determined and the percentage of Cr-release was calculated by using the formula:

$$\%\ \text{Cr-release} = \frac{\text{Radioactivity in the supernatant}}{\text{Total radioactivity (supernatant + pellet)}} - \text{Background radioactivity} \times 100$$

For each value the mean of 4 to 5 wells was used. Controls included spontaneous ^{51}Cr-release, ^{51}Cr-release in the presence of complement alone, antibody alone, normal rabbit serum, and normal rabbit serum together with complement. The activity of the antiserum on normal L-929 cells was determined as well.

The percentage of vaccinia-specific AMC was calculated by using the formula:

$$\%\ \text{Spec. AMC} = \begin{pmatrix}\%\ \text{Cr-release target}\\ \text{cells + antibody + C}\end{pmatrix} - \begin{pmatrix}\%\ \text{Cr-release target}\\ \text{cells + antibody + 5\%}\end{pmatrix}$$

Absorption of H-2 alloantisera. Five-tenths milliliter of anti-H-2^k serum in Eagle's MEM diluted 1/2 was absorbed on 5×10^7 infected enzymatically treated or untreated L-929 cells for 30 min at 37°C. Cells were pelleted by centrifugation 10 min at 3000 rpm, and the cellfree supernatant was carefully removed. The titer of the absorbed serum (supernatant) was determined in a ^{51}Cr-assay: 5×10^4 ^{51}Cr-labeled spleen lymphocytes from C3H mice (haplotype H-2^k) were filled in a volume of 0.05 per well in microplates with 0.05 ml of absorbed and unabsorbed serum diluted in log 2 steps with MEM. After 30 min 0.1 ml of complement was added. After 4 hr incubation at 37°C the percentage of Cr-release was recorded. Lysis was calculated as described in the previous paragraph. The cytotoxic activity of the H-2^k serum reached a plateau of activity between the dilution of 1/16 and 1/64. The mean value of 44.8% cytotoxicity was taken as 100% serum activity.

Cell-mediated cytolysis

Vaccinia virus-specific CMC. The activity of spleen lymphocytes of C3H mice infected 6 days previously with 10^6 $TCID_{50}$ vaccinia virus was tested in an 18-hr Cr-release assay as described earlier (3). Target cells were infected L-929 cells, untreated or treated with different amounts of enzymes. Briefly, 5×10^6 spleen lymphocytes from immunized or normal C3H mice were incubated in microplates with 5×10^4 normal or vaccinia virus-infected, enzymatically treated or untreated ^{51}Cr-labeled L-929 cells in a volume of 0.2 ml at 37°C in a humidified atmosphere with 7% CO_2. Eighteen hours later supernatant and cell pellet were separately removed and Cr-release was calculated. Controls included spontaneous Cr-release (20 to 25% untreated cells, 25 to 35% enzymatically treated cells) and the Cr-release of normal cells in the presence of immune or normal lymphocytes.

The percentage of specific lysis was calculated as follows:

Spec. lysis = % Cr-release by sensitized lymphocytes − % Cr-release by normal lymphocytes

For each value four to five wells were used. Data were subjected to statistical analysis with determination of the mean and the standard error of the mean (S.E.M.) and calculated for significance by the Student *t*-test.

In vitro response against alloantigens. The assay was performed according to the method of Wagner (15). In brief, 1×10^6 mitomycin C-treated C3H spleen cells (stimulator cells) were incubated with 4×10^6 DBA/2 spleen cells (responder cells) in 2 ml Dulbecco's modified medium (GIBCO) supplemented with 2×10^{-5} M mercaptoethanol 10% FCS and 100 μg/ml penicillin and streptomycin. Five or six days later the cells were harvested and their activity on ^{51}Cr-labeled L-929 cells (untreated or treated immediately before with different concentrations of papain) was tested in a 6- to 10-hr assay by using a ratio of 50:1 attacker cells to target cell. The percentage of specific cytolysis was calculated by using the same formula as in the virus specific CMC.

RESULTS

Alteration of surface antigens after enzymatic treatment of target cells. The effect of enzyme treatment on surface antigens of virus-infected L-929 cells was controlled by indirect immunofluorescence (IF). Virus-specific and H-2-specific IF were found reduced or even abolished directly after treatment of cells with proteolytic enzymes. VCN treatment had no effect. Repetition of virus specific IF 4 hr and 24 hr later indicated that viral surface antigens had been resynthesized; immunofluorescence was even intensified on treated cells. H-2-specific IF studies showed that H-2 antigenic sites remained reduced under the experimental conditions.

Vaccinia specific antibody-mediated cytolysis of enzymatically treated cells. To verify the impression given by the immunofluorescence, the activity of an anti-vaccinia serum was tested on vaccinia virus-infected cells treated 4 hr previously with different concentrations of papain, trypsin, pronase, or VCN. Untreated and enzymatically treated infected and uninfected L-929 cells were labeled for 1 hr with ^{51}Cr and then used as targets for AMC. The anti-vaccinia serum showing an anti-complementary activity to a dilution of 1/8 was diluted 1/10 with Eagle's MEM. Guinea pig serum was used as complement source. Specific release of ^{51}Cr did not occur in the presence of normal rabbit serum or when uninfected L-929 cells were used as targets.

As shown in Table II, significant specific lysis of vaccinia virus-infected L-929 cells was observed after enzymatic treatment of vaccinia virus-infected L-929 cells.

Lysis was even increased after treatment with high doses of enzymes, especially in the case of VCN treatment. This demonstrates that viral surface antigens necessary for complement dependent lysis by viral antibodies were resynthesized 4 hr after proteolytic treatment. The increase of specific lysis may be due to an unmasking of antibody or complement-binding sites, to a removal of steric hindrances, or to a change of the surface charge. An enhanced resynthesis of viral surface antigens may be possible as well.

Reduction of H-2 antigenic sites by enzymatic treatment of target cells. To demonstrate resynthesis of viral surface antigens, antibody-mediated cytolysis was sufficient. Absorption studies were performed since AMC does not estimate concentration differences of H-2 antigens on cell surfaces exactly (16).

Equal amounts of infected L-929 cells and infected cells treated with the highest and lowest enzyme concentrations were incubated with an $H\text{-}2^k$ serum at 37°C for 30 min. The absorbed sera were titrated for the amount of residual antibody activity on ^{51}Cr-labeled C3H lymphocytes and compared with the activity of the nonabsorbed serum (Fig. 1).

Treatment of cells with neuraminidase did not alter the capability of cells to absorb the antibody. Almost the same reduction of activity after absorption on treated and untreated cells could be observed. This indicates that antibody-binding sites cannot be removed by neuraminidase. As shown first by Nathenson and Davies (17), the chemical structure of H-2 mouse isoantigens seems to be glycoproteins which contain sialic acid. But the neuraminidase-sensitive sialic acid in the H-2 antigens probably is not involved in the antigenic determinants, since neuraminidase treatment does not affect their activity.

Treatment of cells with proteolytic enzymes causes a reduction of the absorption capacity of the cells. Papain used to disolve H-2 or HL-A antigens for chemical analysis (18) was most effective. No significant amount of alloantibody could be absorbed by cells treated with a high concentration of papain which is reflected by nearly identical slopes of cytolytic activity

TABLE II

Enzyme Concentration	Vaccinia Specific AMC[a] of Infected Enzymatic Treated L-929 Cells			
	Trypsin	Pronase	Papain	VCN
1	18.8 ± 2.0	26.6 ± 1.6	24.6 ± 0.2	13.9 ± 2.4
2	20.2 ± 0.8	27.8 ± 0.4	33.7 ± 1.4	9.3 ± 2.3
3	13.6 ± 3.0	32.9 ± 1.0	24.4 ± 1.8	14.2 ± 2.4
4	16.6 ± 1.9	28.5 ± 1.6	23.4 ± 0.6	15.6 ± 3.6
5	18.6 ± 1.6	28.3 ± 1.2	29.1 ± 2.5	66.8 ± 2.4
Control[c]		13.6 ± 1.8		8.8 ± 1.3

[a] See Table I.

[b] Vaccinia-specific AMC was performed 4 hr after enzymatic treatment of infected L-929 cells in a 4-hr ^{51}Cr-release assay, using overnight infected L-929 cells as targets, guinea pig serum as complement source, and antibody from rabbits after vaccinia virus infection. Spontaneous ^{51}Cr-release of enzymatically treated cells: 20 to 25%.

[c] Control: vaccinia-specific AMC of infected L-929 cells without enzymatic treatment in two different assays for proteolytic enzymes and VCN. Each value represents mean of n = 4 wells ± S.E.M.

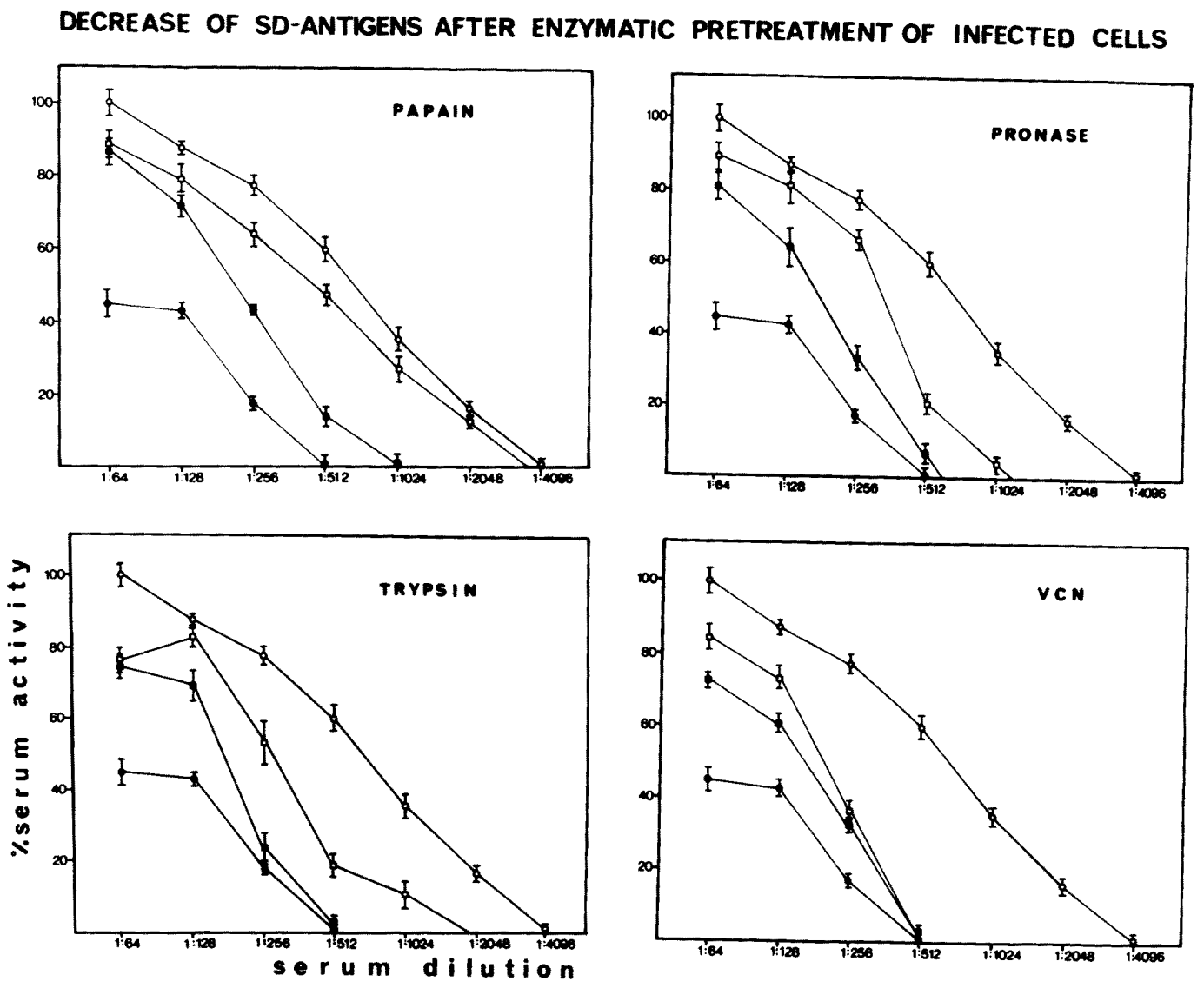

Figure 1. Reduction of H-2 antigenic sites by enzymatic treatment of target cells. The residual cytotoxic activity of an anti H-2^k serum was tested on C3H spleen lymphocytes after absorption on infected enzymatically treated and untreated L-929 cells. Cytotoxic activity of the unabsorbed serum control (O——O), after absorption on infected L-929 cells (●——●), after absorption on infected L-929 cells treated with the lowest (■——■) and highest (□——□) enzyme concentration (see Table 1). The plateau activity of the serum (44.8% ± 3.2%) was taken as 100% serum activity. Data represent the mean of at least four wells ± S.E.M.

shown by control and absorbed serum. Pronase and trypsin in concentrations tolerable for the cells caused a weaker but significant decrease of the ability of the cells to absorb an H-2 antibody.

Alteration of cell-mediated cytolysis by enzymatic treatment of target cells

Virus specific CMC. The cytotoxic activity of virus-specific sensitized lymphocytes was tested on normal and infected L-929 cells treated 4 hr previously with different enzymes. Immune T cells can only lyse syngeneic infected target cells. Allogenic infected or syngeneic noninfected cells are not killed. We got the same results after enzymatic treatment of target cells; neither syngeneic noninfected nor allogenic infected control cells were lysable after enzymatic treatment. Enzymatic treatment had influence only on the lysis of syngeneic infected cells.

Removal of sialic acid resulted in enhanced CMC. Treatment of target cells with proteolytic enzymes diminished the lysis of target cells by the effector cells. Papain treatment was most effective (Fig. 2).

Response against alloantigens. To prove the efficiency of enzymatic treatment on H-2 antigens, L-929 cells treated with papain were used as targets for allogenic CL sensitized against H-2^k. Untreated L-929 target cells were used as controls. Treatment of target cells with different concentrations of papain resulted in the decrease of cytolysis by allogenic killer cells. As uninfected L-929 cells are less fragile than infected L-929 cells, we could use higher concentrations of papain in comparison to virus-specific CMC (Fig. 3).

The enhanced sensitivity of virus-specific CMC to enzymatic treatment suggests an influence of viral infection on H-2 concentration (5) due to blocking of resynthesis and possibly to increased susceptibility of cell membranes for proteolytic treatment. After prolongation of the incubation period of allogenic CMC to 10 hr, no killer cell inhibition was seen, which reflects resynthesis of H-2 antigens during this test period. To compare the resynthesis of target antigens for CMC that means probably H-2 antigens on uninfected and vaccinion

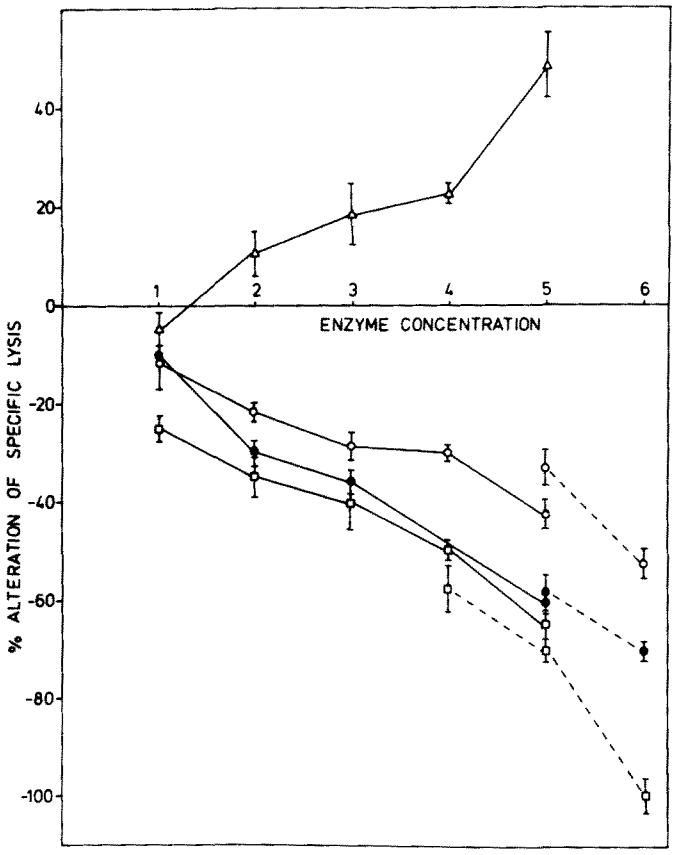

Figure 2. Alteration of cell-mediated lysis after enzymatic treatment of target cells. The action of spleen lymphocytes of C3H (H-2^k) mice sensitized against vaccinia virus and normal control mice was tested on infected L-929 (H-2^k) cells. The figure represents data ± S.E.M. from four independent assays. The 0-line stands for the lysis of untreated infected target cells by virus immune effector cells after subtraction of lysis in presence of normal lymphocytes (specific lysis). The activity of the same effector cells was tested on target cells treated with different concentrations (see Table 1) of enzymes. Δ——Δ, VCN; O——O, O- - - -O, pronase; ●——●, ●- - - -●, trypsin; and □——□, □- - - -□, papain. Some examples of uncorrected values are given to explain the experimental schedule and the transcription of the data into the figure. Control lysis of untreated target cells by NL (normal lymphocytes) 20.3 ± 3.2, IL (immune lymphocytes) 64.3 ± 2.1, □——□ papain concentration *1:* NL 23.1 ± 2.4, IL 57.0 ± 1.2, papain concentration; *3:* NL 21.4 ± 1.3, IL 47.4 ± 3.8, papain concentration; *5:* NL 25.4 ± 0.5, IL 40.7 ± 2.8. O——O, pronase concentration; *3:* NL 28.6 ± 3.4, IL 60.0 ± 2.1. ●——●, trypsin concentration; *3:* NL 26.1 ± 2.8, IL 53.3 ± 1.4.

Alteration of specific lysis was calculated using the formula:

$$\% \text{ alteration} = \frac{\text{specific lysis enz. treated cells}}{\text{spec. lysis of untreated cells}} - 1 \times 100.$$

Differences between lysis of control and enzymatically treated target cells are statistically significant, p-values: Papain concentration. *1:* $p < 0.1$; *3:* $p < 0.001$; *5:* $p < 0.001$; *pronase 3:* $p < 0.1$; *trypsin 3:* $p < 0.01$

infected target cells, the activity of anti-H-2^k and anti-viral CL was tested in a 6- and 16-hr assay on uninfected and infected L-929 target cells pretreated with papain. Untreated target cells were used as controls, (Table III). Uninfected target cells, which were treated with papain, were not lysed in 6-hr assay but in a 16-hr assay, whereas vaccinia virus infected target cells did not regain their ability to be lysed by allogeneic virus-sensitized effector cells or by anti-H-2 killer cells. These data reflect the inhibition of cellular protein synthesis after vaccinia virus infection (19). The serologic (IF) control for H-2 antigens supports the results of CMC.

DISCUSSION

The effects of VCN, papain, trypsin, and pronase on anti-viral AMC and anti-viral CMC were studied. The main results from the present work are that treatment of vaccinia virus-infected target cells for a short period of time with a low concentration of proteolytic enzymes increased AMC and decreased CMC whereas treatment with VCN enhanced both.

Transplantation antigens are re-expressed within 6 hr after removal by proteases on noninfected cells. However, 75% of the antigens are resynthesized 4 hr after enzymatic treatment. Since we used cells infected with vaccinia virus, which inhibits cellular proteinsynthesis (19), no re-expression of cellular surface antigens seemed to take place, which we could show by immunofluorescence, absorption studies, and target cell lysis by anti-alloantigen CTL (cytotoxic T lymphocytes).

The alteration of CMC should be due to the action of enzymes on the target cells. An effect of adherent enzymes on the effector cells seems unlikely. Target cells were used 4 hr after enzymatic treatment and, furthermore, CMC should be enhanced after gentle enzymatic treatment of effector cells as was shown by Kedar *et al.* (20). The fact that the inhibitory activity was reversible in the allogenic control lysis when the incubation period was extended to 10 hr argues against unspecific inhibitory activities of possibly remaining active enzyme concentrations in the test on the effector cells. Treatment of control target cells with more than 10-fold higher enzyme concentrations than in anti-viral CMC had no inhibitory activity on effector cells provided the incubation period was sufficient for re-expression of H-2 antigenic determinants.

Vaccinia surface surface antigens, which are resynthetized after a short time (21), suffice for antibody-mediated cytolysis. The enhancing effect of enzymatic treatment on AMC may be due to increased sensitivity to lysis by antibody and complement or, especially in the case of VCN, to an unmasking of antibody-binding sites (22). From the data showing an inhibi-

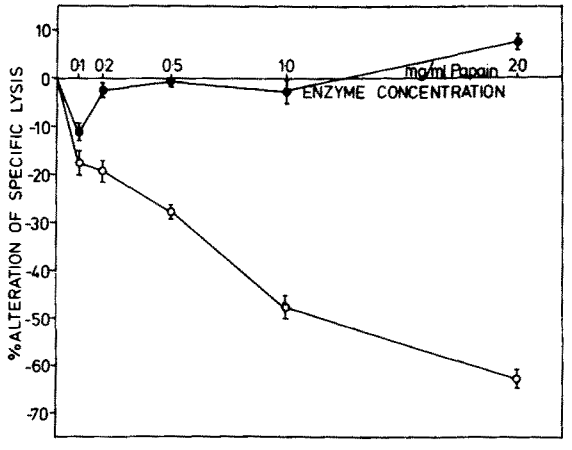

Figure 3. Influence of papain treatment of target cells on cell-mediated lysis by allogenic killer cells. The activity of anti H-2^k killer cells was tested on untreated L-929 cells or cells treated with papain. The data represent the mean of at least three wells ± S.E.M. O——O, CMC tested in a 6-hr assay (specific lysis of control: 56.4%, spontaneous ^{51}Cr-release: 12.5–18.3%); ●——●, CMC tested in a 10-hr assay (specific lysis of control: 63.6%, spontaneous ^{51}Cr-release: 17.5–23.9%). Ratio killer cells to target cells 20:1.

TABLE III

Influence of papain treatment of target cells on CMC by killer cells sensitized against alloantigens or vaccinia virus[a, b]

Effector Cells	IF (H-2-Antigen)[d]	Target Cells			
		L-929	L-929 WR infected	L-929 papain	L-929 WR-infected papain
	After treatment	+	+	−	−
			% Cr-release		
6-hr assay					
Normal DBA/2		18.7 ± 0.8	19.5 ± 1.3	21.3 ± 2.1	22.0 ± 1.3
Normal C3H		19.2 ± 2.1	17.3 ± 3.4	23.4 ± 1.2	22.8 ± 1.8
Anti H-2^k DBA/2		46.3 ± 1.4	35.5 ± 1.8	29.4 ± 4.2[c]	23.1 ± 3.2[c]
Anti-vaccinia WR C3H		21.3 ± 0.8	63.4 ± 2.3	22.1 ± 0.7	26.2 ± 2.8[c]
	After 6 hr	+	+	(+)	−
16-hr assay					
Normal DBA/2		23.5 ± 2.0	22.8 ± 1.2	26.4 ± 2.5	24.7 ± 1.3
Normal C3H		24.5 ± 1.5	23.4 ± 0.6	27.3 ± 3.1	25.9 ± 1.7
Anti H-2^k DBA/2		58.2 ± 2.1	49.3 ± 4.1	61.4 ± 1.6	30.8 ± 1.4[c]
Anti-vaccinia WR C3H		22.4 ± 2.3	82.4 ± 1.9	28.1 ± 2.2	36.2 ± 3.3[c]
	After 16 hr	+	+	+	−

[a] The activity of anti-H-2^k (DBA/2) killer cells (sensitized *in vitro*) and anti-WR (C3H) killer cells (sensitized *in vivo*) was tested in a 6-hr and 16-hr assay on untreated normal and infected L-929 target cells and cells treated with papain (2 mg/ml normal cells and 0.5 mg/ml infected cells).

[b] H-2 specific immunofluorescence was performed immediately after treatment, 6 hr and 16 hr later.

[c] Differences of specific lysis between papain-treated and nontreated target cells are statistically significant: $p < 0.001$.

[d] +, Positive IF; −, negative IF; (+) weakly positive IF.

tion of CMC by antibodies to vaccinia virus-induced antigens on the cell surface (3), one would have expected T cell-mediated lysis of cells lacking H-2 antigenic sites. However, as the only target on the cell surface, virus-specific antigens do not seem to be sufficient for anti-viral CMC. Similar results were presented by R. M. Zinkernagel with the use of virus-infected F9 mouse teratoma cells as targets (41. Cold Spring Harbor Symposium, 1976). Inhibition of CMC by antibody to vaccinia virus is possibly due to steric hindrance.

These results do not support the data which indicate that the presence of haptens on cell surfaces alone can lead to T cell-mediated cytolysis (9). Cytolytic T cells directed to new antigens on cells need, in addition, H-2 antigenic sites on the target cell for their effector function. Reduction of these H-2 targets by destruction with proteolytic enzymes or by covering with H-2 alloantisera decreases the possibility of virus-infected target cells to be lysed by anti-viral CL. This corresponds to observations of *in vitro* lysis of allogenic target cells when a reduction of CMC can be observed after proteolytic treatment of target cells (11). Removal of sialic acid from the target cells enhances their susceptibility to CMC (13). It therefore follows that the CL directed against syngeneic virus-modified target cells acts in an analogous way to the CL directed to alloantigens when the degree of CMC is correlated with the concentration of K or D region gene products on the target cell (16), known to be the main target for allogenic CMC (23).

Viewing the different activities of cytolytic T cells on allogenic cells or syngeneic (probably H-2 altered) cells, which are virus infected, chemically treated, or express H-Y specific gene products (24), it is tempting to assume that the MHC performs a sort of "major rejection complex" (Ceppellini, personal communication). Cytolytic T cells recognize antigenic determinants on cells, and one could speculate that recognition and lysis is possible only via interaction with antigenic expressions of the K or D region of the major histocompatibility complex. T cells interact with various allogenic or modified products of the MHC. New antigenic determinants on cell surfaces which do not interact with K or D structures, should not be targets for cytolytic T cells.

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