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CYTOLYSIS OF NEURAMINIDASE-TREATED AUTOCHTHONOUS LYMPHOID CELLS BY AUTOLOGOUS SERUM

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SUMMARY

Rabbit lymph node cells treated with bacterial neuraminidase become susceptible to cytolysis by autologous antibody and complement. Cytolysis of cells can be prevented by absorbing out the antibodies, probably IgM antibodies, from the autologous serum using neuraminidase-treated autochthonous lymph node cells. The significance of this mechanism is discussed.

INTRODUCTION

We have reported earlier that *Vibrio cholerae* neuraminidase (VCN) treated cells are extremely sensitive to complement (C) mediated lysis (Ray, Gewurz & Simmons, 1971). Autologous serum C shows cytolysis of VCN-treated cells when cobra venom factor (CVF) (Ray, Gewurz & Simmons, 1971) has been used as an activator of C. We report here that autologous rabbit serum, irrespective of the presence of CVF, shows cytolysis of autochthonous lymphoid cells previously treated with bacterial neuraminidase. The cytolytic efficiency of the serum is very much abrogated if the serum is either (a) heated at 56°C for 30 min, or (b) preabsorbed with VCN-treated autologous lymph node cells.

MATERIALS AND METHODS

Lymph nodes from New Zealand white male adult rabbit were taken out, and teased apart in media 199 (M 199) to prepare a single cell suspension (Ray & Simmons, 1971). Cells were labelled with $Na_2{}^{51}CrO_4$ (Ray & Simmons, 1971) and finally suspended in M 199 to have 10^7 cells/ml.

Radiolabelled cells were treated with VCN (25 u/5×10^6 cells/ml), *Clostridium perfringens* neuraminidase (CPN) (25 μg/5×10^6 cells/ml), and influenza virus neuraminidase (IVN)

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(25 u/5 × 10^6 cells/ml) (Ray & Simmons, 1972) at 37°C for 1 hr. After incubation with active or inactive (100°C for 30 min) enzyme, cells were washed sufficiently in an excess of M 199 and diluted to have 10^6 cells/ml. Autologous rabbit serum was prepared in the cold (1°C) following a method detailed elsewhere (Ray & Simmons, 1971).

Treated or untreated cells (10^5) were incubated with autologous serum or heat-inactivated (56°C for 30 min) serum for 30 min at 37°C. CVF (5·8 μg/ml), prepared according to a method described elsewhere (Shin, Gewurz & Snyderman, 1969), was added to it. The incubation was continued for another 30 min at 37°C. Cytotoxicity was determined by measuring the released radioactivity in 1 ml of supernatant (Ray & Simmons, 1971).

RESULTS AND DISCUSSION

In the present communication, the effect of neuraminidase treatment of autochthonous lymph node cells on their susceptibility to cytolysis by autologous serum has been studied. The results are presented in Table 1. Cells treated with VCN and CPN show cytolysis by

TABLE 1. Effect of neuraminidase treatment of rabbit lymph node cells on their susceptibility to undergo cytolysis by autologous serum components

Treatment of cells*	Complement activation†	Lysis (%) (^{51}Cr release)‡
None	CVF	0
VCN	CVF	42
CPN	CVF	23
IVN	CVF	0
VCN	None	40
CPN	None	25
IVN	None	0

* 10^5 cells were incubated with 0·1 ml of autologous serum at 37°C for 30 min and then CVF (0·29 μg in 0·05 ml) or an equivalent amount of media was added and incubated for another 30 min.

† No lysis was observed with heat-inactivated (56°C for 30 min) rabbit serum, or rabbit serum preincubated with CVF (37°C for 30 min) before the addition of cells. The cytotoxic ability of the heated serum could be resumed by the addition of fresh C.

‡ Average of three separate experiments.

autologous serum components; IVN is inactive. The presence of CVF does not affect the cytolysis of VCN-treated cells by autologous serum. It appears that VCN and CPN perhaps can modulate the cell surface in such a way that these cells become sensitive to cytolysis even by autologous serum components. The differential reactivity of neuraminidase obtained from various sources is perhaps due to their differences in the ability to hydrolyse various α-ketosidic linkages between sialic acid and carbohydrate moieties of the cell

surface (Drzeniek & Gaube, 1970; Ray & Simmons, 1973). IVN has been ineffective perhaps because of its inability to hydrolyse 2-6-α-ketosidic linkages between the sialic acid and cell surface mucopolysaccharides (Drzeniek & Gaube, 1970). Therefore, hydrolysis of 2-6-α-ketosidic linkages, as is effectively rendered by bacterial neuraminidase, appears to play a key role to make even cells of autologous origin sensitive to cytolysis by autologous serum. Since VCN has been found to be the most reactive one, in the subsequent investigations only VCN was used.

The cytotoxic factor in the autologous serum appears to be a C-dependent antibody for a variety of reasons: (1) the cytolysis is C-mediated; (2) inactivation of C (56°C for 30 min) is associated with loss of cytotoxicity of the whole serum; (3) the cytotoxicity of the serum has been detected in a fraction collected from Sephadex G-200 column which corresponds to the peak of IgM on this column (unpublished data); (4) the cytotoxic ability of the heated (56°C for 30 min) serum is resumed after the addition of fresh C. Thus it appears that the cytotoxicity of bacterial neuraminidase-treated autologous lymphocytes is mediated by a C-dependent antibody present in the autologous serum.

TABLE 2. Effect of preabsorption of rabbit serum by VCN-treated autochthonous lymph node cells on their ability to show cytolysis of autologous VCN-treated lymph node cells

Number of absorbing cells ($\times 10^6$)*	Lysis (%)† (^{51}Cr release)
None	45
5	30
10	18
25	0
50	0

* 25×10^6, untreated, autochthonous rabbit lymph node cells could not show any appreciable reduction in the cytolysis when used for absorption.

† Values represent the average of six separate experiments.

It can be argued, however, that this naturally occurring antibody may be directed against any bacterial antigens which are associated with the neuraminidase preparation as contaminants. Those antigens may have been absorbed onto the lymphocyte surface facilitating the antibody to bind to the cell surface to effect cytolysis. However, this possibility does not seem to exist for the following reasons: (1) cells treated with heat-inactivated bacterial neuraminidase (100°C for 30 min) do not show cytolysis with autologous serum; (2) cells treated with bacterial neuraminidase in presence of the negative feed-back inhibitor of the enzyme, i.e. *N*-acetyl neuraminic acid (5 mg/ml), does not show lysis with the autologous serum.

If the lysis of bacterial neuraminidase-treated cells by autologous serum components is due to a C-dependent autoantibody, this antibody should bind with VCN-exposed newly

available antigen on the cell surface. Investigations have, therefore, been carried out to see if this naturally occurring autoantibody can be absorbed out by VCN-treated autologous lymph node cells.

Radiolabelled rabbit lymph node cells were treated with VCN, washed, and their viability counted. Cells were diluted in M 199. Aliquots containing various numbers of cells ($1–50 \times 10^6$ cells) were added in different tubes and centrifuged (300 *g* for 5 min). Undiluted autologous rabbit serum (0·1 ml) was added to tubes containing VCN-treated or untreated cells and incubated at 37°C for 1 hr. Unabsorbed sera were titrated for the presence of autoantibody against VCN-exposed antigenic determinants. Radiolabelled and VCN-treated rabbit lymph node cells were used for this study. Results are presented in Table 2. 25×10^6 VCN-treated lymph node cells can completely absorb out the naturally occurring autoantibody from the autologous serum. However, the same number of untreated cells are quite ineffective. It is possible that VCN-treated cells might have absorbed out at least some C components (Dalmasso & Müller-Eberhard, 1964), while absorbing out those autoantibody molecules. Depletion of both these serum factors might have resulted in the decrease in the amount of cytolysis (Table 2).

From the above results it appears that bacterial neuraminidase renders cells susceptible to immune destruction even by 'self' immune components. It is evident that autoantibody against 'self' cellular antigens which are normally hidden or masked, does exist in the system. At times these antigens may become exposed, thus facilitating the reaction with the autoantibody and resulting in the destruction of 'self' cells.

It is not known, however, if any physiological alteration with respect to cell surface sialic acid may lead to immune destruction of 'self' cells *in vivo* following the same mechanism as detailed above. Diseased, aged, damaged, or transformed cells may often exhibit changes associated with the exposure of 'hidden or crippled' antigens. A possibility, however, exists that similar mechanism may have some relevance to the immune clearance of defective or aged cells, thus maintaining a dynamic stability of cell population in the individual.

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