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SHEEP ERYTHROCYTE SENSITISATION AND RESTRICTION TO LYSIS BY THE COMPLEMENT SYSTEM FROM EQUINE AND SHEEP SERUM

SENSIBILIZAÇÃO DE HEMÁCIAS DE CARNEIRO E RESTRIÇÃO À LISE PELO SISTEMA COMPLEMENTO DO SORO EQÜINO E DE CARNEIRO

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SUMMARY

The effect of the degree of sheep red cell sensitisation by antibody in the resistance to lysis by sheep and horse complement was analysed. The results showed that, as the level of sensitisation increased, so did the homologous complement activity, eventually leading to one hundred percent of lysis; the same was observed for horse serum, generally considered as not efficient at lysing these cells. Therefore, the homologous and heterologous restriction can be overridden by this treatment. This may be of interest for the comprehension of the mechanisms involved in the modulation of the restriction phenomenon in health an in diseases involving complement in domestic animals and in human, as well as in laboratory diagnosis where hemolytic assays are used for measurement of complement activity and evaluation of presence of antibodies in serum samples.

UNITERMS: Lysis; Erythrocytes; Antibodies; Hemolysis

INTRODUCTION

Complement is not efficient at lysing erythrocytes from the same species. Membrane proteins regulate the C3 convertase[7,12] and the membrane attack complex (MAC)[17,19], restricting complement activity. Erythrocytes from individuals affected by paroxysmal nocturnal hemoglobinuria are defficient in Decay Accelerating Factor (DAF)[13,14], Homologous Restriction Factor (HFR)[5,18], and CD59[8], and are susceptible to homologous complement. Resistance to complement action is also observed in heterologous systems, depending on the cell and of species of complement. As an example, horse complement is inefficient to lyse sheep red cells[2,11,12], and control of the MAC is involved[11]. However, this restriction appears to be temporary. Storage *in vitro* increases the susceptibility of sheep red cells to horse complement[1], and human cells to human complement[10]. This may partially explain opposing results in the literature[9,15], and ilustrates the importance of studies analysing possible factors involved in this phenomenon.

In studies employing sheep serum, inconsistent results were observed using different lots of the same monoclonal antibody to sensitise sheep red cells[1]. The level of cell sensitisation is known to interfere in the degree of lysis, but its correlation to the homologous (and heterologous) restriction to complement has not been focalized. Here sheep erythrocytes were sensitised with variable amounts of antibody and subjected to lysis by sheep (homologous) and horse (heterologous) serum; guinea pig and human serum were used for comparison. The results are discussed in terms of degree of restriction presented by these sensitised cell to complement action. The importance of studies on this subject is reinforced considering situations of occurrence of high levels of antibodies and cell sensitisation *in vivo*[16], the cytolitic reactions by homologous complement[4], and its role in immune surveillance and disease.

MATERIAL AND METHOD

Complement Fixation Diluent (CFD) containing Ca^{2+}, Mg^{2+} and 0.1% gelatin; and phosphate-buffered saline (PBS) were prepared as described[6]. Two aliquots of blood were collected under sterile conditions from each adult healthy sheep: in 4% sodium citrate for the cells, and without anticoagulant for the serum. Human, horse and guinea pig sera were prepared from blood of adult healthy individuals, pooled and stored at 70ºC. When necessary, sera were absorbed at 0ºC with sheep red cells. Rat monoclonal (IgM) anti-sheep red cell antibody was supplied by the MRC Centre, Cambridge, England. Polyclonal (mainly IgG) anti-sheep red cell antibody was prepared by injecting rabbits with cell stroma, collecting serum, and inactivating it at 56ºC for 30 minutes. Hemolytic

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assays were performed incubating 40 µl of EA with serum diluted 1:2 in CFD to 1.0 ml. After 30 minutes of incubation at 37°C, and centrifugation, lysis was determined by the absorption of the supernatant at 412 nm. Percentages of lysis were calculated considering the absorption of EA lysed in distilled water as 100%. Dilutions of monoclonal antibody used to EA preparation offered approximately 8.4 x 10^3 - 5.4 x 10^5 molecules of antibody/cell.

RESULTS AND DISCUSSION

Sheep erythrocytes from one animal were washed in PBS and sensitised with various concentrations of antibody (Erythrocyte-Antibody complex, EA) according to HARRISON; LACHMANN[6] (1986), washed 3 times in CFD, and resuspended to a concentration of 1.7 x 10^8 cells/ml. The EA were incubated at 37°C with sheep serum from the same animal or horse serum from a pool, and lysis was measured after 30 minutes. The results of a representative from 4 assays are shown in Fig. 1. At adequate antibody concentration, the sheep serum lysed one hundred percent of the autologous cells, and the same occurred for horse serum. Fresh cells were used to abrogate age-related variations in susceptibility to complement[1]. Therefore, homologous and heterologous restriction to lysis by complement can be overridden by this treatment. Similarly, reactive lysis by homologous complement was observed when the number of C5b67 sites was sufficiently increased, compensating the lysis inhibition[17]. The effect was similar with both monoclonal and polyclonal antibodies, indicating that it is not specific to a particular antibody or, for the polyclonal antiserum, to blockage of restricting factors on the cell membrane. The use of one serum dilution throughout the experiment, and the absence of lysis by inactivated sera (not shown) eliminate the possibility of interference of factors other than complement in the results.

Data in Tab.1 (representative from 4 assays) there is a comparison of lysis of EA by sheep, horse, human and guinea pig complement, clearly showing the high efficiency of human and guinea pig serum in this system. However, similarly to data in Fig. 1, sheep and horse serum diluted 1 in 2 lysed the cells at the appropriate antibody concentration. In this assay, the use of antibody diluted only 1 in 32 or 1 in 64 would lead conversely to the conclusion that the activity of respectively sheep or sheep and horse complement is absolutely restricted by sheep red cells.

Lysis by homologous complement *in vitro* was observed to human red cells sensitised by antibody[10], however it peaked at levels of 40-60%, with routinely lower values (less than 35%). Our results complement these observations showing that, one hundred percent of homologous lysis occurs *in vitro* since cells are heavily sensitised. Thus, the level of sensitising antibody affects the restriction presented by cells to complement attack and may have an important role in defense and disease mechanisms. High sensitisation of cells may occur *in vivo* in some cases leading to severe hemolysis or cell death. This is ilustrated by the occurrence of cytolytic reactions by homologous complement, such as destruction of cat cells infected with feline leukemia virus by cat anti-tumor IgG and cat complement[4]; or severe hemolysis associated with pathological processes where high levels of antibodies and cell sensitisation occurs *in vivo*, as in cold agglutinin syndrome in humans[16]. Apart from this, they may have interest also for the routine use of sensitised sheep erythrocytes in hemolytic assays for laboratory diagnosis involving determination of antibody titres or complement levels; particularly considering here the large domesticated animals, whose sera show low lytic efficiency against these cells[3], and the possibility of improvement of assay sensitivity by increasing the level of cell sensitisation.

The mechanism by which high levels of antibody sensitisation overcome homologous restriction is not clear. It is possible that enhanced rates of complement activation may allow MAC formation to occur sufficiently rapid to avoid the action of the restriction proteins. Further investigation of the mechanism is therefore in progress.

CONCLUSIONS

Our results show that the level of cell sensitisation affects homologous (and heterologous) restriction presented by cells to complement attack. This may help the comprehension of the mechanisms underlying the modulation of the restriction phenomenon as well as its role in determining complement action in defense and diseases. In addition, or using hemolytic assays for research and laboratorial diagnosis, one has to be aware that antibody titres may vary depending on the complement source used in the titration assays, and that in some cases test sensitivity for measuring complement levels can be improved by increasing cell sensitisation. This may be important in veterinary pathology since, as previously stated, serum from most common large domestic animals do not lyse sheep cells, and there is, in consequence, little information on their complement level in health and in disease[3].

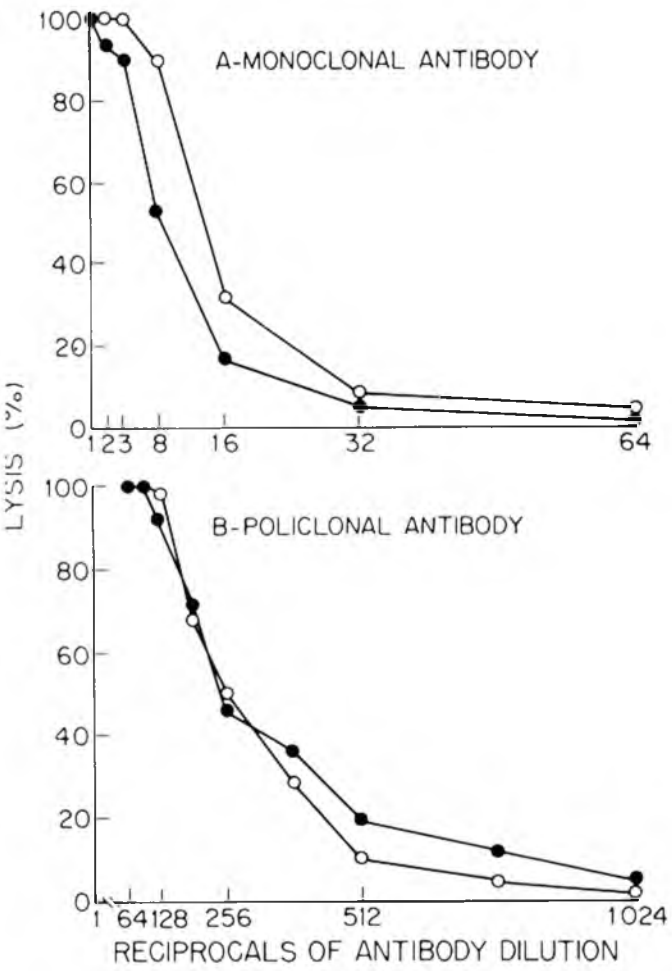

FIGURE 1

Effect of the degree of cell sensitisation by antibody in the lysis of sheep EA by sheep (O) and horse (O) sera.

EA were incubated at 37°C for 30 minutes with diluted serum. After centrifugation, lysis was determined by the absorption of the supernatent at 412 mm, considering the absorption of EA lysed in distilled water as 100%.

TABLE 1

Percentage of lysis of sheep EA (monoclonal antibody) by sheep, horse human or guinea pig serum*. Ribeirão Preto - SP, 1993.

Reciprocals of antibody dilutions / Complement Source**	2	4	8	16	32	64
human	100.0	100.0	100.0	100.0	100.0	100.0
guinea pig	100.0	100.0	100.0	100.0	100.0	100.0
equine	100.0	100.0	100.0	97.6	26.1	0.0
sheep	100.0	88.4	52.9	14.9	0.0	0.0

* The hemolytic assay was preformed as in Fig. 1.

** Complement was diluted 1:2 in CFD (see methods for diluent composition).

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RESUMO

Neste trabalho foi analisado o efeito do grau de sensibilização de hemácias de carneiro por anticorpo, na resistência à lise pelo complemento do soro eqüino e de carneiro. Os resultados mostraram que, à medida que houve um aumento do nível de sensibilização, ocorreu também um aumento da atividade do complemento homólogo, levando a 100% de lise; o mesmo foi observado para o complemento do soro eqüino, geralmente considerado ineficiente na lise destas células. A restrição à lise pode, assim, ser sobrepujada por este tratamento. Estes dados podem ser de interesse para a compreensão dos mecanismos envolvidos na modulação do fenômeno de restrição, na saúde e em doenças envolvendo o sistema complemento em animais domésticos e no homem, bem como em diagnóstico laboratorial, onde são utilizados ensaios hemolíticos para medida de atividade do complemento e pesquisa de anticorpos em amostras de soro.

UNITERMOS: Lise; Eritrócitos; Anticorpos; Hemólise

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