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DIFFERENT PHENOTYPES OF COMPLEMENT RECEPTOR ON HUMAN ERYTHROCYTES[1]

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Summary. Complement receptor on human erythrocytes was detected by haemagglutination assay with aggregated IgG and quinea pig complement. On the basis of the pattern of haemagglutination produced by erythrocytes from various donors, three different phenotypes of complement receptor were distinguished. High phenotype corresponded to strong haemagglutination, an intermediate one — to weak haemagglutination, and low phenotype occurred in cases with undetectable haemagglutination. Radioimmunobinding results proved that the intensity of haemagglutination corresponded to the complement receptor sites density on human erythrocytes.

Complement receptor on human erythrocytes is similar to that found on lymphocytes, neutrophiles and monocytes (Fearon 1980). The receptor recognizes C3b, a larger fragment of activated C3 complement component. Recently, Wilson et al. (1982) using ^{125}I-labeled anti-C3b receptor antibodies presented evidence indicating that the number of C3b receptors on human erythrocytes is genetically regulated and is determined probably by two codominant alleles. The present study showed that the haemagglutination assay can be used for distinction of erythrocyte complement receptor phenotypes. Therefore this simple technique can be applied to study genetic variability of C3b receptor on human erythrocytes.

MATERIAL AND METHODS

Blood samples were taken from 171 healthy individuals (118 men and 53 women) between 18-45 years of age. Twice washed erythrocytes were tested for C3b receptor using haemagglutination assay according to the method described by Miyakawa et al. (1981). Briefly, serial two-fold dilutions of 25 µl of aggregated IgG (initial concentration 50 µg/ml) in Hanks balanced solution (HBS) supplemeted with 0.5% bovine serum albumin were prepared in U-bottom polystyrene microtitre plates (Sterilin, England). Then 25 µl of guinea pig serum diluted 1:60 was added to each well, and the plates were incubated at 37°C for 40 min. After incubation, 25 µl of 2-mercaptoethanol solution (5 mg/ml) was added to each well to protect the genera-

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ted C3b sites from decay. Subsequently, 25 µl of 1% erythrocyte suspension was transferred to each well. The plates were incubated for further 2 hr at room temperature, then the haemagglutination was assessed. C3b receptor on erythrocytes was also determined by radioimmunobinding technique according to the method described by Tsuda et al. (1979) for the detection of immune complexes in serum. 10^8 erythrocytes (25 µl) were incubated with human aggregated IgG (10 µg/ml) and guinea pig complement as in haemagglutination assay. Thereafter, to the cells washed three times, 25 µl/25 µg of ^{125}I-labeled rabbit anti-human IgG (Marchalonis 1969) was added and incubated for 30 min at 4°C. Then the erythrocytes were washed four times and the bound radioactivity was counted.

RESULTS AND DISCUSSION

The haemagglutination titre that is, the highest dilution of aggIgG that produced agglutination, was usually 1 : 2560 to 1 : 5120, which corresponded to aggIgG concentration of 1.6 — 0.8 µg/ml. There was only a slight difference in haemagglutination titre among erythrocytes of the studied donors. However, it was found that the intensity of haemagglutination significantly varied among normal individuals. Erythrocytes from all individuals studied were classified according to haemagglutination intensity as strongly positive, weak positive and negative. The pattern of haemagglutination was characteristic for the studied subjects and did not change over the period of 6 months. Radioimmunobinding technique was applied to test whether the haemagglutination intensity was due to different number of the C3b receptor sites on erythrocytes. The obtained results (Table 1)

Table 1. Determination of the erythrocyte C3b receptor by haemagglutination test and radioimmunobinding technique

Haemagglutination intensity	Radioimmunobinding c.p.m.	N
Strong	4216±865	4
Weak	1902±734	4
No agglutination	472±287	4

indicated that the erythrocytes producing strong haemagglutination bound much more agg-IgG-C3b complexes than the erythrocytes which were negative in the haemagglutination test. Therefore, it can be concluded that the characteristic pattern of haemagglutination corresponded to the density of the C3b receptors on human erythrocytes. This finding justified the distinction of three different phenotypes of erythrocyte C3b receptor. High phenotype corresponded to strong haemagglutination, an intermediate to weak haemagglutination, and low phenotype produced no haemagglutination. The incidence of the erythrocyte C3b receptor phenotypes in a population sample is presented in Table 2. Further studies are needed to establish the frequency of gene for high and low expression of complement

Table 2. Frequency of erythrocyte complement receptor phenotypes in normal men and women

Phenotype	Phenotype frequencies (%)	
	men (n=118)	women (n=53)
High haemagglutination (homozygote C3b receptor (+))	63.6	79.2
Intermediate haemagglutination (heterozygote C3b receptor (±))	31.4	18.9
Low haemagglutination (homozygote C3b receptor (−))	5.0	1.9

receptor on human erythrocytes. This may be of some clinical significance in the light of reports by Siegel and Gleicher (1981) and Iida et al. (1982), demonstrating the defective complement receptor on erythrocytes from patients with systemic lupus erythematosus and cancer.

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RÓŻNE FENOTYPY RECEPTORA DLA KOMPLEMENTU NA LUDZKICH ERYTROCYTACH

Streszczenie

Receptor dla komplementu na ludzkich erytrocytach wykrywano testem hemaglutynacji z użyciem zagregowanej IgG i komplementu świnki morskiej. Na podstawie intensywności hemaglutynacji z erytrocytami od różnych dawców wyodrębniono trzy fenotypy receptora

dla komplementu: wysoki, odpowiadający silnej aglutynacji, pośredni dający słabą hemaglutynację oraz niski fenotyp w tych przypadkach, w których erytrocyty nie uległy widocznej aglutynacji. Wyniki testu radioimmunologicznego potwierdziły, że stopień intensywności hemaglutynacji odpowiada gęstości miejsc receptorowych dla komplementu na ludzkich erytrocytach.

РАЗНЫЕ ФЕНОТИПЫ РЕЦЕПТОРА ДЛЯ КОМПЛЕМЕНТА НА ЭРИТРОЦИТАХ ЧЕЛОВЕКА

Резюме

Рецептор для комплемента на эритроцитах человека был обнаружен тестом гемаглютинации с помощью агретатированного IgG и комплемента морской свинки. На основании интенсивности гемаглютинации с эритроцитами от разных доноров было выделено три фенотипа рецептора для комплемента: высокий, соответствующий сильной аглютинации, средний, дающий слабую аглютинацию, и низкий фенотип в тех случаях, в которых эритроциты не подвергались видимой аглютинации. Результаты радиоиммунологического теста подтвердили, что степень интенсивности гемаглютинации соответствует плотности рецепторных мест для комплемента на эритроцитах человека.