Monospecific Antibody Plaque Formation by Spleen Cells from Mice Immunized with Sheep and Chicken Erythrocytes

It is still not clear whether an individual immunologically competent lymphoid cell can form antibodies to single or multiple antigens[1,2]. In attempts to clarify this point, several groups of investigators have resorted to the laborious and time-consuming micro drop procedure[1-5]. Most of the experimental data obtained to date suggests that a majority of antibody forming cells apparently produce only one, or at the most, two unrelated antibodies to bacterial or viral antigens. The present report is concerned with use of the newly described localized hemolysin procedure[6-8] to determine whether or not lymphoid cells from mice immunized with a mixture of two unrelated red cell antigens can hemolyze both cell types simultaneously.

In the method developed by JERNE[6,7], lymphoid cells from animals injected with foreign erythrocytes are incubated in semi-solid agar containing a dilute suspension of the erythrocytes used for immunization, followed by incubation with guinea-pig complement. Distinct zones of hemolysis ('antibody plaques') are formed in the agar-red cell plate. In the experiments reported here, spleen cells from mice injected with sheep and chicken erythrocytes were incubated in agar containing either one or the other red blood cells, or a mixture of both. For a typical experiment, 0.5 ml of 20% washed red blood cells was injected intraperitoneally into NIH mice as follows: group A mice received sheep erythrocytes only, group B received chicken erythrocytes only, group C received a mixture of both erythrocytes. Four days later, blood samples were obtained by retro-orbital puncture for agglutinin and hemolysin titrations. Mice injected with both red cells formed relatively high titers to both RBCs. Mice injected with either one or the other formed detectable circulating antibody only to the RBCs used for immunization.

Cell suspensions were prepared from each group by 'teasing' spleens in sterile Hanks solution, pH 7.2, containing 20% normal calf serum and excess antibiotics[9]. The cell suspensions were filtered through gauze, washed three times with sterile Hanks solution, and resuspended at a concentration of 1 to 5×10^6 cells per ml. For the plaque assay, 0.1 ml suspension was rapidly mixed with 2.0 ml of 0.7% melted Noble agar (maintained at 48–50°C) containing 1 mg DEAE-dextran and 0.1 ml 10% suspension of either sheep, chicken, or mixed erythrocytes. The warm cell-agar mixture was carefully layered over a 3 mm thick base of solidified 1.4% agar in 100 mm diameter petri dishes and permitted to solidify. The plates were incubated at 37°C for 1 h, then treated with 5 ml guinea-pig complement, diluted 1:10, and reincubated for an additional 30 min. Plates were examined macroscopically and microscopically for localized zones of hemolysis. The number of plaques per million cells plated was recorded and the proportion of completely clear plaques to cloudy plaques containing either unlysed large elliptical cells (apparently chicken RBCs) or unlysed smaller round cells (apparently sheep red cells) was determined.

The Table indicates results of a typical series of experiments in which spleen cells were tested for plaque forming ability with individual and mixed red cells. Spleen cells from group 'C' mice formed numerous plaques when incubated either with sheep, chicken, or mixed RBCs. Significantly more plaques were observed with mixed RBCs as compared to plates with sheep or chicken cells only. However, all plaques in mixed plates were markedly hazy and much less distinct than those in plates containing only one red cell type. Microscopic examination invariably revealed only partial lysis. There were either many unlysed sheep cells or many unlysed chicken cells per plaque. Macroscopic and microscopic examination of several thousand plaques in numerous mixed plates with spleen cells from doubly immunized 'C' mice did not reveal a single plaque containing completely lysed red cells.

Plaques obtained with spleen cells from control 'A' or 'B' mice injected only with one RBC were generally

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Antibody plaque formation per 10^6 viable nucleated spleen cells from NIH mice injected with either sheep or chicken erythrocytes or a mixture of both and tested in agar plates containing either individual or mixed erythrocytes

Test group	Erythrocyte antigen injected (5×10^9 RBCs i.v./mouse)	Mean peak hemolysin titer[a]	Test erythrocyte in agar plate					
			Sheep		Chicken		Sheep and chicken	
			No. of plaques	% clear[b]	No. of plaques	% clear[b]	No. of plaques	% clear[b]
A	Sheep	S, 1:2035 C, 1:<10	422	95.3	3	67.7	390	0
B	Chicken	S, 1:<10 C, 1:1650	2	0	286	80.4	216	0
C	Sheep plus chicken	S, 1:1965 C, 1:1508	378	94.7	302	92.7	635	0

[a] Tube dilution hemolysin titer of serum specimens obtained 4 days after immunization. [b] Macroscopically sharp, well defined zones of hemolysis; microscopically few unlysed RBCs in plaque. S = anti-sheep RBC titer; C = anti-chicken RBC titer. 6 to 10 mice per group. Average of 5 experiments.

completely clear. Plaque formation was quite specific; mice injected with sheep cells formed plaques only with sheep RBCs. Mice injected with chicken cells formed plaques only with chicken RBCs. However, plaques with chicken cells were somewhat less distinct than those with the sheep RBC system.

In several preliminary experiments it has been observed that mice receiving multiple injections of mixed sheep and chicken erythrocytes over a period of several weeks yield spleen cell suspensions which produce occasional completely clear zones of hemolysis in agar containing mixed sheep and chicken erythrocytes[10]. However, immunization over a prolonged period of time with either RBC alone results in appearance of serum hemolysins to the heterologous erythrocytes.

In contrast to the tedious and time-consuming microdrop technique utilized by others, the hemolysis plaque method has permitted rapid sampling of relatively large numbers of antibody producing cells from immune mice. The results obtained suggest that lymphoid cells capable of simultaneously lysing two red blood cell types in a single antibody plaque are either absent or quite scarce in spleen cell suspensions from mice receiving a single injection of mixed sheep and chicken erythrocytes[11].

Zusammenfassung. Milzzellenaufschwemmungen von Mäusen, die mit einem Gemisch von Schaf- und Hühnererythrocyten einmal injiziert wurden, verursachen lokalisierte, hämolytische Zonen (antibody plaques), die nicht aus den beiden Zelltypen, sondern entweder nur aus Schaf- oder nur aus Hühnerzellen bestehen. Lymphatische Zellen, die gleichzeitig zwei verschiedene Hämolysine abscheiden können, sind in Milzzellenaufschwemmungen von zweifach immunisierten Mäusen anscheinend abwesend oder nur in ganz geringer Anzahl vorhanden.

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[10] H. Friedman, in preparation.

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Über eine mangelnde Parallelität zwischen Ionenpumpenhemmung und Kontraktionskraftsteigerung

Die Ionenpumpenaktivität der Erythrocytenmembran kann durch Herzglykoside gehemmt werden (Harris[1]; Kahn und Acheson[2], Kahn[3]; Machowa[4]). Diese Wirkung wird in einen kausalen Zusammenhang mit der positiv inotropen Wirkung der Glykoside gebracht (Förster und Sziegoleit[5], Schatzmann[6]). Da Pharmaka bekannt sind, die die Kontraktionskraft steigern, ohne die Kaliumaufnahme von Kälteerythrocyten zu vermindern, z. B. Adrenalin und die Veratrumalkaloide (Kahn und Acheson[2]), teilen wir im folgenden den Befund mit, dass verwandte Substanzen mit gleicher Hemmwirkung auf die Ionenpumpenaktivität der Erythrocytenmembran die Kontraktionskraft des Herzens gegensätzlich beeinflussen.

Bei unseren Untersuchungen von Guanylhydrazonen, die uns freundlicherweise von Herrn Prof. G. Kroneberg (Farbenfabriken Bayer) zur Verfügung gestellt wurden, prüften wir die Wirkung folgender Substanzen auf die Kontraktionskraft isolierter Meerschweinchenvorhöfe und die Kaliumakkumulation von Kälteerythrocyten derselben Spezies (s. Formeln).

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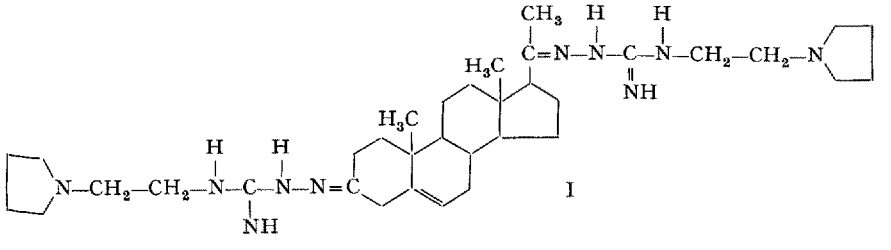

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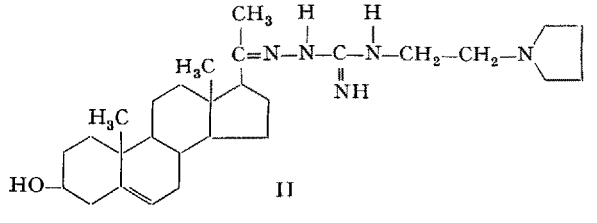

II

I. Δ5-Pregnen-3,20-dion-N,N'-äthylpyrrolidinbisguanylhydrazon.

II. Δ5-Pregnen-3β-hydroxy-20-on-N,N'-äthylpyrrolidinmonoguanylhydrazon.