The Use of Complexes of Lymphocyte Antigens with Antibody as Immunogens for the Preparation of Antilymphocytic Sera

Antilymphocytic sera (ALS) contain antibodies which react against components other than lymphocytes, and some of these antibodies are toxic[1-3]. Less toxic sera have been obtained using subcellular antigens, chiefly membranes[2,4,5]. The possibility of using nontoxic sera to isolate purified antigens by immunoprecipitation seems attractive, although recovery of the antigen from the antigen-antibody complex may be difficult. We have therefore investigated the possibility of using undissociated antigen-antibody complexes as immunogens in the preparation of ALS. This approach may seem unlikely to yield potent ALS, since specific antibody tends to depress the immune response to antigen, whether the antibody is present as a result of prior immunization or is administered passively[6]. In some instances[6], however, antibody has an adjuvant effect when administered simultaneously with antigen. The regulatory effect of antibody on the immune response has been reviewed by UHR and MOLLER[6].

In a preliminary experiment, to determine whether or not immunosuppressive antibody could be obtained using a complex as immunogen, whole mouse thymocytes were agglutinated using a horse anti mouse thymocyte serum. As the cell suspension was treated with increasing amounts of antiserum contaminating erythrocytes agglutinated first. These were removed by centrifugation, resulting in a considerable purification of the antigen. At higher antibody levels the thymocytes agglutinated and were collected by sedimentation. The agglutinated cells were washed 3 times in physiological saline and injected into rabbits. The rabbits (No. 543 and 544) received 1 injection of 10^8 cells in Freund's Complete Adjuvant (FCA) in the foot pad, followed 14, 21, 28 and 35 days later by i.v. injections given without adjuvant, and were bled out on day 46. The in vitro titres and the median survival time in the mouse skin allograft test, shown in the Table, indicate that the sera were of moderate potency.

In a second experiment 2 rabbits (No. 572 and 573) were injected with an antigen prepared by precipitation of a thymocyte lysate using a rabbit anti mouse thymocyte membrane serum. The antiserum was prepared as described elsewhere[4] and was absorbed with glutaraldehyde-fixed mouse erythrocytes. The mouse thymocytes were lysed by homogenization in hypotonic buffer[4] and the lysate was centrifuged at 100,000 *g* for 30 min to sediment the particulate elements. The pellet was resuspended in a small volume of saline and treated with the antiserum, which agglutinated some of the particles. The agglutinated material was separated from unagglutinated fragments by low-speed centrifugation, resuspended in saline, and injected into rabbits. Two control rabbits (No. 574 and 575) received membrane prepared from the same lysate by density gradient centrifugation, as described previously[4]. The rabbits received two injections (day 0 and 14) in FCA in the foot pad, and were bled out on day 21. The results set out in the Table indicate that 'membrane antigen' separated by precipitation with antibody was as effective as membrane prepared by physical separation methods. The graft survival times were somewhat superior in the antisera raised against complex, and the in vitro results indicated that the sera raised against complex and those raised against membrane were very similar in their range of biological activities.

To investigate the behaviour of complexes of human cells, human peripheral lymphocytes were agglutinated with a rabbit antiserum raised against a soluble antigenic extract from human lymphocytes[5]. The agglutinated cells were washed and injected into rabbits 552 and 553, with the same regime as that used for rabbits 543 and 544. The in vitro results shown in the Table indicated that the sera

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In vitro[a] and in vivo[b] testing of antisera

		HA[a]	LA[a]	PA[a]	Cytox.[a]	IMF[a]	RI[a]	MST[a]
1st Experiment	543 Mouse thymocyte complex	1:48	1:24	>1:2	1:64	1:32	1:8000	24.0
	544 Mouse thymocyte complex	1:24	1:32	>1:2	1:64	1:64	1:4000	23.5
2nd Experiment	572 Mouse thymocyte lysate complex	1:16	1:64	>1:2	1:128	1:32	1:4000	32.5
	573 Mouse thymocyte lysate complex	1:16	1:96	>1:2	1:128	1:64	1:4000	30.0
	574 Mouse thymocyte membrane	1:4	1:48	>1:2	1:128	1:64	1:8000	32.5
	575 Mouse thymocyte membrane	1:32	1:64	>1:2	1:96	1:64	1:2000	20.0
3rd Experiment	552 Human blood lymphocyte complex	1:192	1:128	>1:2	1:16	1:64	1:8000	Not tested
	553 Human blood lymphocyte complex	1:192	1:16	>1:2	1:4	1:32	1:4000	Not tested

[a] Haemagglutination (HA), leucocyte agglutination (LA), platelet agglutination (PA) and cytotoxicity (Cytox.) tests were carried out as described elsewhere[8]. None of the sera tested gave any detectable platelet agglutination at the lowest dilution used (1:2). The rosette inhibition test (RI) was carried out essentially as described by BACH et al.[7] and the immunofluorescence (IMF) test as described by THOMAS et al.[8].
[b] The mouse homograft test was carried out as described previously[4]. The median survival time (MST) for groups of 6–10 mice is given in days. The standard deviation around the mean survival time was 2–6 days for the experiments described here. Control mice injected with normal rabbit serum instead of ALS gave MST 12–13.5 days.

would be non-toxic (low haemagglutination and platelet agglutination) and of moderate potency in vivo. These experiments indicate that lymphocyte antigen-antibody complexes are immunogenic. Our results also suggest that by precipitation with an antiserum raised against cell membranes we have selected a fraction of the cellular antigens which leads to more potent and less toxic antisera than are obtained against whole cells. A small amount of antiserum raised against highly purified membrane or soluble antigen[2,4,5] could be used to prepare enough complexed antigen to immunize several large animals, and the antigen need not be recovered from the complex. Further immunological procedures which might prove useful in the preparation of ALS are illustrated by the sequential agglutination of erythrocytes and thymocytes in the first experiment and by the prior absorption of the agglutinating antiserum with an immunoabsorbent prepared from erythrocytes in the second experiment.

Résumé. Des complexes antigène-anticorps préparées par réaction du sérum antilymphocytaire (ALS) avec des lymphocytes ou avec des broyats lymphocytaires furent injectées aux lapins. Les ALS obtenu ainsi des lapins furent immunodépresseurs. Cette technique peut être utilisée pour obtenir des ALS plus specifique et moins toxique.

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Heme Synthetase in Thalassemia

Heme synthetase (HS) is the mitochondrial enzyme which introduces iron into protoporphyrin and thereby participates in the last stage of heme synthesis. A clinically useful test for measurement of HS activity on cell lysates was recently described by Bottomley[1] and closely followed in our laboratory. The test is based on the progressive incorporation of radioactive iron into heme, using optimal amounts of protoporphyrin and ferrous iron as substrates. The increase of specific radioactivity of extracted and crystallized heme[2] is linear during the first 30 to 60 min, and the incorporation of radioactivity may therefore be expressed per minute and number of cells originally present. The current investigations were performed on peripheral blood rather than bone marrow. After removal of plasma and the buffy coat, HS activity of washed and lysed red cells was measured. Prior to hemolysis red cells and reticulocytes were counted.

First we examined blood from 8 hematologically normal, healthy donors. The incorporation of radioactivity into heme per min and 5×10^9 red cells, including about 1.8% reticulocytes, was approximately 0.04% of initial radioactivity added. In contrast, incorporated radioactivity reached 0.5% in the blood of these same normal individuals when we attributed the HS activity to 10^9 reticulocytes. Both values appeared normal to markedly increased in the blood obtained from 6 patients with thalassemia major, and the measured activities were more variable (Table).

The ratio of iron incorporation into heme attributed to 10^9 reticulocytes (R) over that calculated per 5×10^9 red cells, including reticulocytes in the percentages indicated (E), again was more variable in the thalassemia group. A decrease in the ratio would indicate that relatively more HS activity resides with red cells which have matured beyond the stage of the reticulocyte, i.e. HS activity remains longer with maturing red cells or, alternatively and more likely, the proportion of young red cells is increased.

Our results do not reflect mechanisms controlling heme synthesis by normal and thalassemic reticulocytes or red cells. Heme synthesis is indeed influenced by the availability of the protein moiety of hemoproteins[3], although the mechanism of reduction of heme synthesis in thalassemia is not well understood[4]. Earlier studies of Steiner et al.[5] on HS of bone marrow suggested that this enzyme also was reduced in thalassemia. However, HS values expressed per number of bone marrow erythroblasts include the pool of ineffective erythropoiesis in this disorder. Our present results indicate that HS activity of *circulating* red cells and/or reticulocytes of thalassemic blood is normal or increased, rather than reduced as a consequence of impaired globin synthesis[6].

Zusammenfassung. Hämsynthetase (HS)-Aktivität zirkulierender Retikulozyten und Erythrozyten von Normalspendern und Patienten mit Thalassämie wurde in vitro bestimmt. HS war normal oder erhöht bei Thalassämie. Jugendliche, über das Stadium des Retikulozyten hinaus gereifte Erythrozyten enthalten wahrscheinlich noch einen signifikanten Anteil der im peripheren Blut messbaren HS-Aktivität.

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Heme synthetase activity of normal and thalassemic hemolysates

^{59}Fe incorporated into heme/min/number of cells (%)	Normal donors (mean ± standard deviation)	Thalassemia (range)
5×10^9 erythrocytes (E)	0.037 ± 0.017	0.09 — 4.0
10^9 reticulocytes (R)	0.530 ± 0.244	0.70 — 7.3
reticulocyte number	av.: 1.8%	1 — 14%
ratio *R/E*	av.: 14.3	1.3 — 20.4

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