Effects of testosterone on lymphoid tissues and immune responses

Group	Wet weight (mg/10 g body wt.)		PFC/10^6	RFC/10^6	Haemolysin titer ($\log_2$)
	Thymus	Spleen			
1	7.7 ± 0.2[a]	47.0 ± 3.3	389 ± 63	$6{,}667 \pm 552$	7.3 ± 0.7
2	3.1 ± 0.4	38.7 ± 0.4	95 ± 13	$4{,}098 \pm 480$	4.3 ± 0.3
3	0.9 ± 0.2	36.1 ± 2.3	32 ± 10	$3{,}249 \pm 392$	4.0 ± 0.2
4	0.9 ± 0.2	37.8 ± 4.2	126 ± 15	$5{,}822 \pm 874$	4.6 ± 0.3

[a] Each value represents the mean $\pm$ standard error of 3 mice.

logical examination revealed that the thymus, spleen and mesenteric lymph node of testosterone-treated animals (Figures 1, 2 and 3b) were severely depleted in lymphocytes compared with the normal appearance in control animals (Figures 1, 2 and 3a). Severe depletion of lymphocytes was observed not only in the thymus-dependent areas[5] but also in the thymus-independent areas. Germinal centers were markedly decreased in number. The development of their envelope or cap of small lymphocytes was markedly supressed, even if a few of germinal centers appeared in the thymus-independent areas. These changes were most conspicuous in group 3. Plasma cells were abundantly distributed, as in normal animals. It was of interest that the red pulp of testosterone-treated animals showed an extensive increase in myeloid elements, more marked in the erythroblastic series than in the granulocytic and megakaryocytic series.

Discussion. Severe depletion of lymphocytes was observed histologically in the thymus-dependent and thymus-independent areas of the spleen and lymph node of testosterone-treated animals. This suggests that testosterone inhibits the differentiation of both thymus-derived and bone marrow-derived lymphocytes[5,6]. The involution of the thymus by testosterone has been reported by previous investigators[7–9]. However, no histopathological changes following testosterone treatment have been reported previously in the peripheral lymphoid tissues. Erythropoiesis was observed to predominate in the spleen of animals treated with testosterone. Erythropoietic activity of testosterone is well known[10,11]. BATCHELOR[12] speculated that the administration of testosterone would encourage differentiation of certain stem cells in the direction of the erythroid series at the expense of their differentiation towards the population of lymphocytes which migrate to the thymus.

Zusammenfassung. Nachweis, dass die Behandlung letal bestrahlter und mit isologem Knochenmark regenerierter Mäuse mit Testosteron zu starker Herabsetzung der immunologischen Reaktivität und zu eindeutiger Reduktion des lymphatischen Gewebes führt.

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Some Immunochemical Studies on Leukocyte Antigens in Acute Leukemia

Many investigations also carried out various immunological studies on human leukemia. Most of these investigations support the idea that leukemic cells have a specific antigen[1–3]; others found that some of leukemic antigens were lost during disease[4–10]. The purpose of the present work was to detect antigenic differences between normal and leukemic human leukocytes.

Material and methods. Human leukocytes from patients with acute leukemia were injected to rabbits and their antigenicity was studied by Ouchterlony's immunodiffusion, immunoelectrophoresis and complement-fixation test.

Preparation of antigens. a) Leukemic and normal leukocytes were collected by the 6% dextran sedimentation method and washed 3 times with physiological

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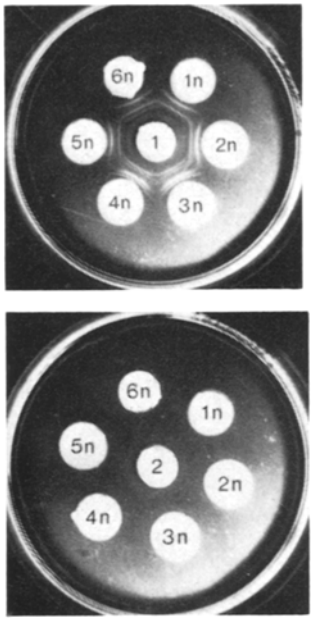

Fig. 1 and 2. Antigenicity of human normal leucocyte lysates against serum obtained against normal leukocytes (1) and serum obtained against leukemic leukocytes (2).

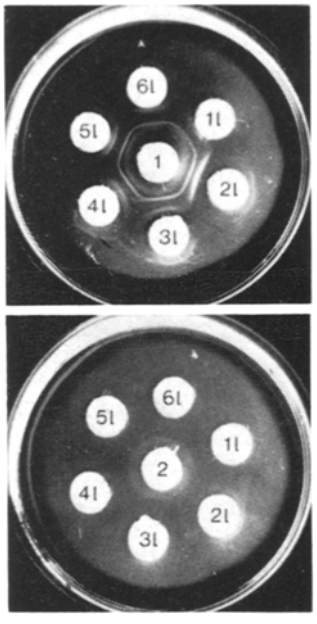

Fig. 3 and 4. Antigenicity of human leukemic leukocyte lysates against serum obtained against normal leukocytes (1) and serum obtained against leukemic leukocytes (2).

saline solution and used as antigens[11–13]. b) Leukemic and normal platelets were prepared by DUCKERT's method[14]. c) Leukemic and normal erythrocytes were washed 10 times with physiological saline solution.

Preparation of antisera. The first group of 10 rabbits were immunized with leukemic leukocytes, and the second group with normal leukocytes. The cycle of immunization was carried out in a month's time, 4 times, at an interval of 7 days, with increasing protein content of leukocyte suspensions and different ways of application.

Absorption procedures. The rabbit antisera for immunochemical studies were absorbed with human plasma, erythrocytes and platelets. For the complement-fixation test, the same antisera were cross absorbed in addition with normal and leukemic leukocytes. Agar gel diffusion after Ouchterlony's method. Immunoelectrophoresis after the method of HYDE, GARB and BENNETT[3]. Complement-fixation test: The antisera obtained were cross-titrated with arbitrarily chosen 5 normal and 10 leukemic leukocyte lysates, before and after the proceeding of cross-absorptions with normal and leukemic leukocytes, by the micromethod in cold of the complement-fixation test.

Results and discussion. By diffusion of 40 normal leucocyte lysates against the serum obtained against

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Table I. A complement-fixation test of the serum obtained against normal human leukocytes with normal and leukemic leukocyte lysates

Quantity serum against normal leukocytes in 1 ml saline	Normal leukocyte lysates					Leukemic leukocyte lysates								
	Before absorption													
	1n	13n	20n	33n	40n	11	41	171	191	261	271	281	291	351	381
0.10	++++	++++	++++	+++±	++++	++++	+++±	±	++++	++±	++++	++++	+	+++	+++
0.08	+++	+++±	+++	+++	+++	++±	++	–	+++	+	++±	+++	±	++	+±
0.06	++±	+±	++	++	+	+±	+	–	++	±	+	++	+	+	±
0,05	+	±	+	±	–	±	–	–	+	–	–	+	–	–	–
from 0.05 until 0.005	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	After absorption with leukemic leukocyte lysate													
0,10	+±	+	++	+	+±	–	–	–	–	–	–	–	–	–	–
0.08	+	±	+	±	+	–	–	–	–	–	–	–	–	–	–
0.06	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
from 0.05 until 0.005	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

+, hindrance of haemolysis; ++++, full hindrance of haemolysis; –, full haemolysis.

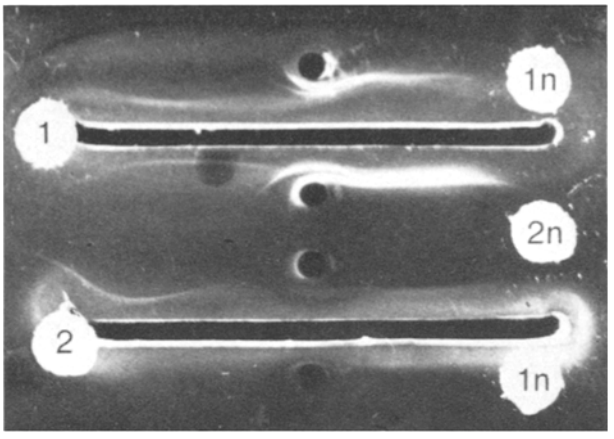

Fig. 5. Antigenicity by immunoelectrophoresis of human normal leukocyte lysates against serum obtained against normal leukocytes (1) and serum obtained against leukemic leukocytes (2).

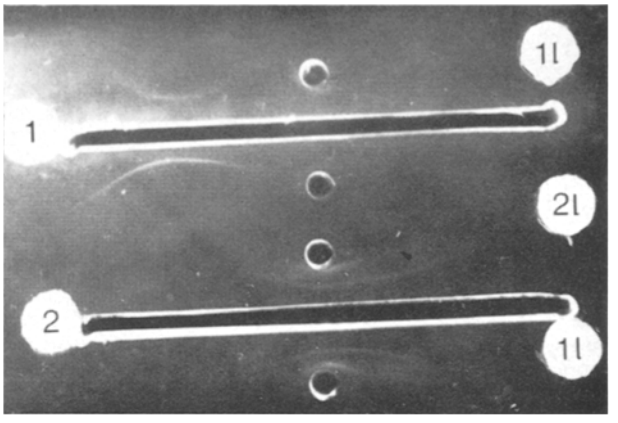

Fig. 6. Antigenicity by immnoelectrophoresis of human leukemic leukocyte lysates against serum obtained against normal leukocytes (1) and serum obtained against leukemic leukocytes (2).

normal human leukocytes, the formation of 4–6 precipitin lines was observed. Between the same cell lysates and the serum obtained against leukemic leukocytes only 1 precipitate from each was formed (Figures 1 and 2).

By diffusion of 40 leukemic leukocytes lysates against serum obtained against leukemic leukocytes the form of 1 precipitin line was observed (by leukocytes of 4 patients 2 precipitates each and by leukocytes of 2 patients 3 precipitin lines each). The same lysates formed 2–5 precipitin lines against the serum obtained against normal leukocytes (Figures 3 and 4).

The data obtained by immunoelectrophoretic study showed that by normal leukocyte lysates 205 electrophoretic fractions are being formed against serum produced against normal leukocytes, in comparison to 72 fractions against the serum obtained against leukemic leukocytes. Thus their fractions against a normal rabbit antiserum were distributed uniformly in the zones of moving of γ-, β_2-, β_1-, α_2- and α_1-globulins while those against leukemic antiserum showed a tendency for moving with a speed corresponding to the speed of moving of γ- and β_2-globulins (Figure 5).

The immunoelectrophoresis of leukemic lysates showed 154 electrophoretic fractions in comparison with 277 of normal leukocyte lysates. The electrophoretic fractions of leukemic lysates against serum obtained against normal leukocytes were disposed mainly in the zones of migration of β_1- and α_2-globulins while their fractions against a leukemic antiserum moved like γ-, β_2- and β_1-globulins (Figure 6).

The method of complement-fixation test confirmed the results obtained by immunochemical techniques. The cross-absorptions showed that the serum obtained against leukemic leukocytes is thoroughly absorbed by normal leukocyte lysate, while the leukemic lysate is not able to absorb all antibodies in the serum obtained against normal leukocytes (Tables I and II).

In order to interpret the results obtained by methods of immunodiffusion, immunoelectrophoresis and complement-fixation test, a work hypothesis was made that the leukocytes from 40 investigated patients with acute leukemia have lost a part of antigens building up the antigenic profile of normal leukocytes. By this hypothesis, part of the experimental data were explained. As a result of the loss of antigens leukemic leukocytes formed a smaller number of precipitin lines (electrophoretic fractions) against serum obtained against leukemic leukocytes in comparison with precipitin lines which normal leukocyte lysates formed against the serum obtained against normal leukocytes.

Table II. A complement-fixation test of the serum obtained against leukemic human leukocytes with normal and leukemic leukocyte lysates

Quantity serum against leukemic leukocytes in 1 ml saline	Normal leukocyte lysates				Leukemic leukocyte lysates										
	Before absorption														
	1n	13n	20n	33n	40n	11	41	171	191	261	271	281	291	351	381
0.10	+++	++±	++++	++	++++	++++	+++	±	++++	+++	++++	+++	+	++	++±
0.08	++	+	+++	+	+++	++	±	−	++	+	++	++	±	+±	++
0.06	±	−	++	±	+±	+	−	−	+±	−	+	+	−	−	±
0.05	−	−	±	−	±	−	−	−	±	−	±	−	−	−	−
from 0.05 until 0.005	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	After absorption with normal leukocyte lysate														
from 0.10 until 0.005	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

+, hindrance of haemolysis; ++++, full hindrance of haemolysis; −, fully haemolysis.

The analyses that have been made in detail while obtaining rabbit antisera could explain the rest of the experimental results. The sera against normal leukocytes were obtained through injection of mixed donor's leukocytes. The material used for immunization of another group of animals contained 97–100% paramyeloblasts. It was supposed that the established antigenic reduction did not touch, in the same degree, the elements of the white blood line of the patients investigated. Probably the antigen loss was most strongly expressed by the paramyeloblasts. It was admitted that under the influence of the leukemic agent from the antigenic profile of the paramyeloblasts a large part of the antigens was lost. Consequently the sera obtained against the paramyeloblasts did not contain all the antibodies with which the antigens of the remaining types normal and leukemic leukocytes would be connected.

Résumé. Les antigènes leucémiques de 40 malades atteints de leucémie aiguë et de 40 adults sains ont été étudiés à l'aide des sérums de lapin et par des méthodes d'immunodiffusion, d'immunoélectrophorèse et la réaction de fixation du complément. On a trouvé que les leucocytes de malades étudiés ont perdu une part des antigènes observés dans les leucocytes du groupe de contrôle des personnes normales.

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Studies on Phytohemagglutinins XXI. The Covalent Oligomers of Lysozyme — First Case of Semisynthetic Hemagglutinins

Lysozyme (3.2.1.17 mucopeptide N-acetylmuramoylhydrolase), is known to contain a combining site for linear oligosaccharides formed by N-acetyl-D-glucosamine[1,2]. In this respect it resembles the wheat germ agglutinin[3]. Similar sugar specificity is exerted also by hemagglutinins from other sources, e.g. the phytohemagglutinin II of the furze seeds (*Ulex europaeus* L.)[4] and the phytohemagglutinin from potato tubers (*Solanum tuberosum* L.)[5].

At present the assumption seems to be well justified[6] that the minimum of two sugar-combining sites in the lectin is necessary for the agglutinating activity. Recent findings[6] show that an increasing number of the combining sites in a lectin molecule, as effected by polymerization of the lectin, results in an increased hemagglutinating activity. All these facts imply that artificial preparation from monovalent molecules of molecules with more sugar-binding sites should give rise to semisynthetic agglutinins from nonagglutinating substances capable of binding carbohydrates.

In the present work lysozyme was polymerized by the action of glutardialdehyde[7] in an attempt to prepare a semisynthetic model agglutinin mimicking the action of phytohemagglutinins.

Materials and methods. A 1% water solution of glutardialdehyde (10 µl) was added to 100 mg of lysozyme (3 times crystallized chicken egg white lysozyme, Nutritional Biochemical Co., Cleveland, Ohio, USA) dissolved in 4 ml of 0.1 *M* phosphate buffer, pH 7.2. The mixture was intensively stirred for 1 min and then allowed to react for 24 h at room temperature. A small amount of a precipitate which formed was removed by centrifugation and the yellow supernatant was applied to a Sephadex G-100 column (2 cm × 200 cm) in saline (0.9% NaCl solution). Elution was effected by saline at a rate of 12 ml/h. Fractions of 4 ml were collected and the absorbance at 280 nm was measured.

Hemagglutinating activity was assayed by the serial dilution procedure, using a 2% saline suspension of the thrice washed erythrocytes. After 30 min at room temperature, the tubes were centrifuged for 1 min at 1000 rev/min (centrifuge Janetzki T 12) and observed macroscopically.

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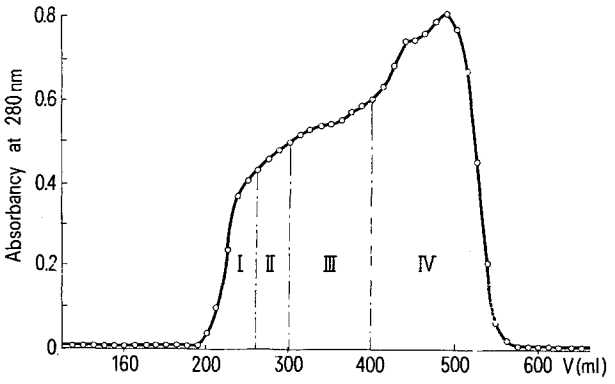

Fig. 1. Gel filtration of lysozyme oligomers on a Sephadex G-100 column (2 cm × 200 cm) in 0.9% NaCl. See text for details.

Erythroagglutinating activity[a] of lysozyme oligomers

	Yield (mg)	Erythrocytes				
		A_1	A_2	B	0	Rabbit
Original reaction mixture	90	16	16	16	16	0
Fraction I	5	128	128	128	128	0
Fraction II	11	32	32	32	32	0
Fraction III	32	2	2	2	2	0
Fraction IV	40	0	0	0	0	0
Lysozyme	—	0	0	0	0	0

[a] Expressed in serial dilution titers of 1% protein solutions.