Immunoprecipitation of tadpole (*Rana catesbeiana*) and frog labelled haemolysates

Antigen	TCA precipitable (cpm)		Antiserum	Immunoprecipitated (cpm)	
	^{14}C	^{3}H		^{14}C	^{3}H
Tadpole haemolysate (^{3}H)		648 (100%)	Anti-T	—	246 (38%)
			Anti-F	—	20 (3%)
			Control	—	18 (3%)
Frog haemolysate (^{14}C)	191 (100%)		Anti-T	10 (5%)	—
			Anti-F	174 (91%)	—
			Control	6 (3%)	—
Tadpole (^{3}H) and frog (^{14}C) haemolysates	100 (100%)	286 (100%)	Anti-T	27 (27%)	123 (43%)
			Anti-F	96 (96%)	132 (46%)
			Control	7 (7%)	16 (6%)

Anti-T and anti-F refer to antisera of rabbits immunized with the major components of tadpole and frog haemoglobins, respectively. The control consisted of serum of a non-immunized rabbit.

A conceivable explanation for this finding could be that roughly half of the amount of tadpole or frog haemoglobins in the mixture are present in the form of tadpole-frog haemoglobin complexes or hybrids. Haemoglobin is known to undergo dissociation in acid or alkaline solutions, concentrated salt media[6], and even at neutral pH and low ionic strength[7]. Hybridization i.e., recombination of subunits belonging to different mammalian[8] or amphibian[2] haemoglobins, has been reported to occur only when mixtures of haemoglobins were exposed to a pH near 4.7 or 11.0 and subsequently neutralized. The experiments reported in this paper were carried out at pH 8.3 and at low ionic strength. If valid, the conclusion that *R. catesbeiana* tadpole and frog haemoglobins form hybrids or complexes under these conditions, would suggest possible differences in the association-dissociation properties of haemoglobins from different species.

Résumé. Des anticorps spécifiques de l'hémoglobine de la grenouille et têtard de *Rana catesbeiana* ne peuvent pas être distingués lorsque tous deux sont présents dans la mixture de la réaction. Cette observation peut être expliquée par la formation d'hybrides entre les hémoglobines adultes et larvaires de *Rana catesbeiana*.

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Inhibition by Testosterone of Immune Reactivity and of Lymphoid Regeneration in Irradiated and Marrow Reconstituted Mice

It is well known that testosterone given to the chick embryo prevents the development of lymphoid tissues in the bursa of Fabricius and inhibits the antibody formation[1,2]. The present investigation was undertaken to determine the effect of testosterone on the antibody response in mammals.

Materials and methods. Male C3H/He mice of 8 weeks old received 850 rad whole body γ-irradiation from a ^{60}Co source and were then injected i.v. with 36×10^6 syngeneic bone marrow cells within 3 h. The animals were divided into 4 groups. The 1st group of animals was not treated with testosterone. 10 mg of testosterone in 0.2 ml aquous suspension (Enarmon, Teikokuzoki Pharm. Co., Tokyo; a mixture of isotonic saline, 17β-hydroxy-androst-4-en-3-one, arabic gum and thimerosal) was given s.c. 7 times to the 2nd group at 4 h intervals; and to the 3rd group at 24 h intervals from the day of marrow reconstitution. The 4th group animals were injected also 7 times at 24 h intervals from the 6th day after marrow reconstitution. The animals in all groups received i.v. 2×10^8 sheep red blood cells 30 days after marrow reconstitution and were sacrificed 5 days after antigen challenge. Assays of haemolysin were carried out, employing microtitration equipment. The plaque forming cells (PFC) were enumerated according to the method of Cunningham and Szenberg[3]. The rosette forming cells (RFC) were calculated using the method of Haskill et al.[4]. The sections of the thymus, spleen and mesenteric lymph node were stained with methyl green pyronin.

Results. The results are shown in the Table. The involution of the thymus was striking in testosterone-treated animals. The reduction of the spleen weight was not so remarkable. The fall of the PFC response was particularly conspicuous in group 3. The haemolysin titer also fell markedly. The fall of the RFC response was not so dramatic as that of the PFC response. The histo-

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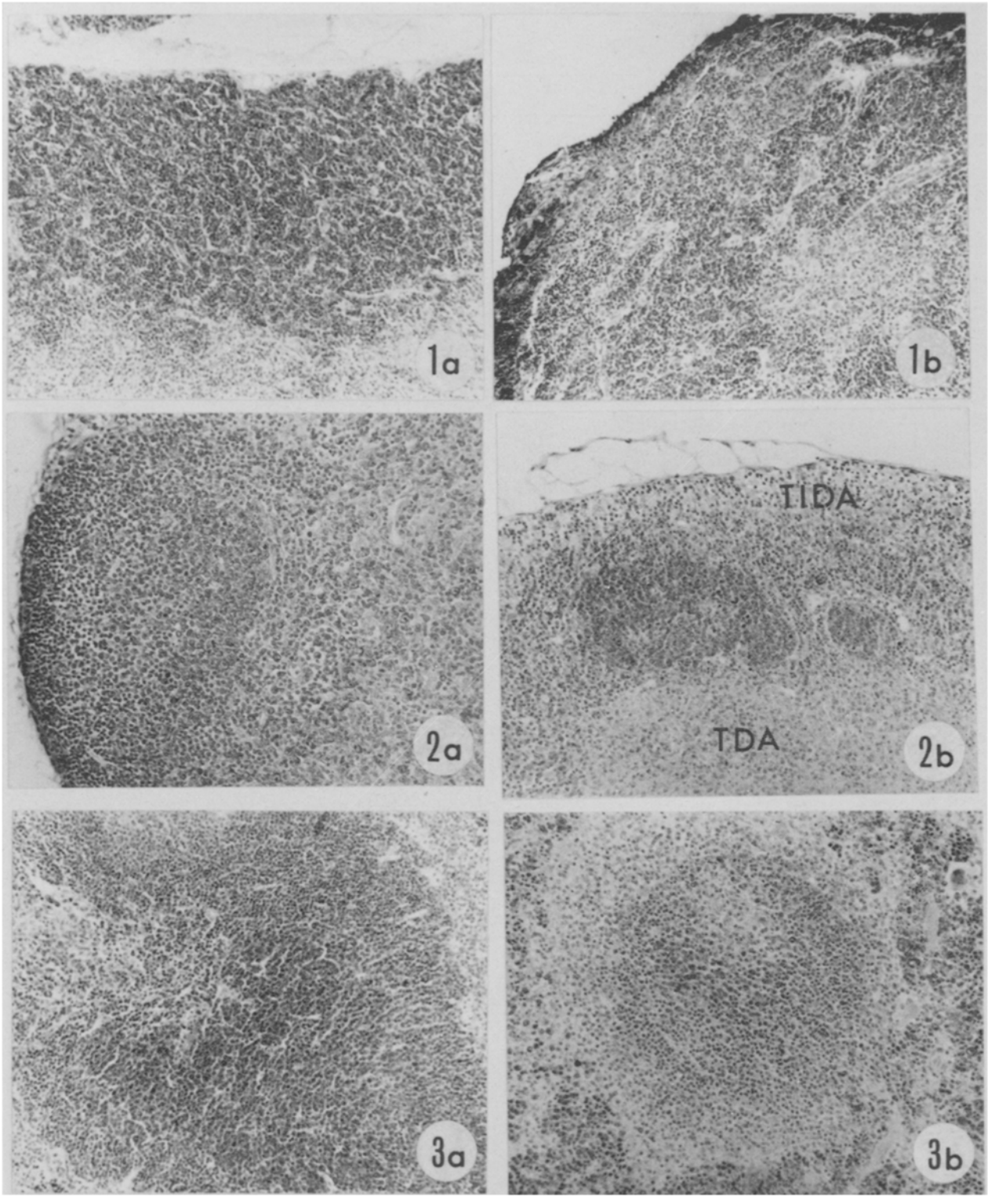

Fig. 1–3. 1. Thymus. Note a striking atrophy in b) in contrast to the normal appearance in a). a) nontreated b) treated with testosterone 7 times daily from the day of irradiation and marrow reconstitution (group 3). 2. Mesenteric lymph node. In b) severe depletion can be seen not only in the thymus-dependent paracortex (TDA) but also in the thymus-independent superficial cortex (TIDA). Note the 'bare' germinal center in b) in contrast to the germinal center enveloped with compact small lymphocytes in a). 3. Spleen. The development of the white pulp and the germinal center is far less in b) than in a); in contrast, erythropoiesis in the red pulp is more predominant in b) than in a). 2a) ×180, the others ×110.

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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