harvesting time of 24 h 100% of pig PBL mitoses were 1st mitoses[20]. According to this kinetic pattern of division in PHA-stimulated pig lymphocytes it is clear that the cells of category IV, that means cells with 3 or more clusters of stained areas, are mainly cells involved in 2nd mitoses. In addition to this clearcut common trend, some differences were established between PHA- and ConA-activated PBL on one hand and PW-activated cells on the other. The distribution of stained NORs in the PW-stimulated cells differs significantly from that in the PHA- and ConA-stimulated cells. This difference expressed in metaphase is not unexpected in consideration of the facts, that PHA and ConA stimulate a larger spectrum of T-cell, and PW a larger spectrum of B-cell, subpopulations. Therefore expression of rRNA-synthesis in metaphase cells seems to vary between certain T- and B-cell subpopulations. The early increase of silver staining in lectin stimulated cultures reveals the reactivation of the rRNA-cistron as an early event in lectin-induced lymphocyte activation.

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Erythropoietic stimulation enhances, and erythropoietic inhibition suppresses, multidirectional differentiation in 5-day transient endogenous spleen colonies

W. Wiktor-Jedrzejczak[1]

Department of Radiation Immunology, Military Institute for Hygiene and Epidemiology, ul. Szaserow 128, PL-00-909 Warsaw (Poland), 14 November 1980

Summary. Transient endogenous spleen colonies were found to be composed of either erythroid, granuloid or megakaryocytic cells, or mixtures of these cell types. Independently of the directions of differentiation of the colonies their formation was uniformly stimulated by bleeding and almost completely prevented by hypertransfusion. It is suggested that cells which form these colonies constitute a separate class of pluripotential hemopoietic progenitors, whose differentiation in either direction passes the stage sensitive to erythropoiesis-modulating factors.

Transient endogenous spleen colonies are formed during hemopoietic regeneration of the mouse following midlethal doses of irradiation[2]. They began to appear on day 3 postirradiation, their number reaches a maximal level on day 5, and by day 7 they disappear from the spleen through the release of mature cells into the circulation[3]. Later, beginning from days 8-9 postirradiation they are followed by the 2nd and final wave of regeneration derived from hemopoietic stem cells, i.e. spleen colony forming units (CFU-S)[3]. The cells that form transient colonies (transient endogenous colony forming units (TE-CFU)), were shown to be non-transplantable; they had extensive proliferative potential and were considered to be early erythropoietic progenitors in nature[4]. This notion was supported by the observation that the number of TE-colonies formed was greatly increased by erythropoietin injection or postirradiation bleeding and was decreased in response to hypertransfusion. Additionally, it was supported by the predominantly erythroid composition of colonies[4]. In a previous study we observed that a considerable proportion of these colonies is in fact granulopoietic[5]. At this time we suggested that similarly to erythropoiesis, granulopoietic progenitor cells may also form these colonies[5]. In order to continue this line of investigation we attemted to repeat the experiments of Gregory et al.[4] along with detailed morphological examination of colonies formed. It was expected that postirradiation bleeding would have a selective stimulatory effect on TE-CFU erythropoiesis. On the other hand, hypertransfusion would selectively inhibit red cell formation, while TE-CFU granulopoiesis and (if any) megakaryopoiesis would be either unaffected or might even be increased in plethoric mice in agreement with reported observations[6,7].

Unexpected findings from the experiments outlined above constitute the basis of this report.

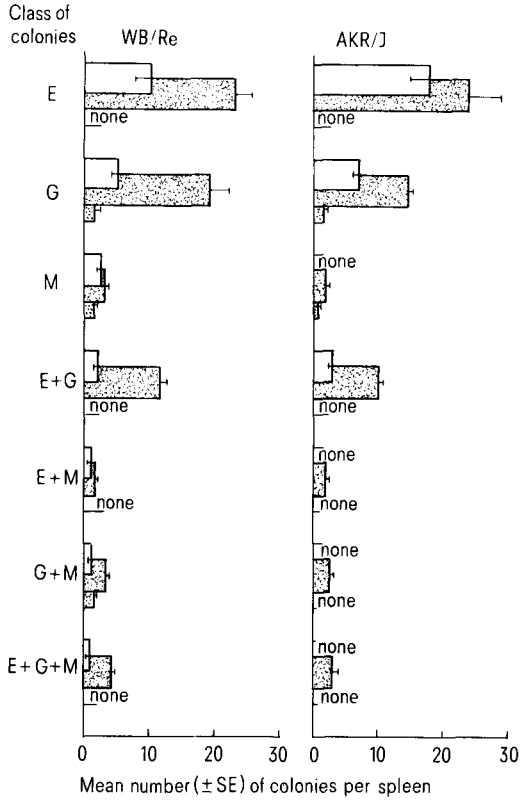
Materials and methods. WB/Re and AKR male mice, 8-12 weeks old, were obtained from the Animal Facility, Postgraduate Center, Military School of Medicine, Warsaw, Poland. Groups of 10 mice were X-irradiated with 350 rad using a Medicor THX-250 unit, operated at 125 kV, 20 mA, 0.5 mm Cu added filtration to give a dose rate of 64 rad/min. These mice were either made polycythemic prior irradiation, or bled within 4 h postirradiation or did not undergo any treatment except for irradiation. 5 days fol-

The effect of bleeding and hypertransfusion on the total number of macroscopic and microscopic* 5-day endogenous spleen colonies formed following irradiation of mice with 350 rad X-rays

Strain	Manipulation in addition to X-irradiation	The number of macroscopic 5-day endogenous colonies per spleen** ($\bar{x} \pm SE$)	The number of microscopic 5-day endogenous colonies per spleen** ($\bar{x} \pm SE$)
WB/Re	None	36.2 ± 3.6	22.5 ± 6.2
	Bleeding	83.3 ± 4.5***	57.4 ± 4.4***
	Hypertransfusion	No macroscopically visible colonies	5 ± 2.2***
AKR	None	27.8 ± 3.8	29.0 ± 4.9
	Bleeding	63.0 ± 3.9***	53.3 ± 6.6***
	Hypertransfusion	No macroscopically visible colonies	2.8 ± 1.1***

* As counted on median sections of spleens only; ** 10 mice per each group; *** significance level $p < 0.001$.

lowing irradiation mice were sacrificed, their spleens were removed and placed in Tellysniczky's fixative and the number of colonies on the spleen surface was counted as previously described[4,5,8]. Subsequently, spleens were processed for histology and microscopic evaluation was performed on median sections using the criteria of Silini et al.[9] as previously described[8]. Briefly, the following morphological types of colonies were distinguished: erythropoietic (E), granulopoietic (G), megakaryopoietic (M), and 4 types of mixed colonies; E+G, E+M, G+M, and E+G+M. When appropriate, mice were bled from retroorbital plexus and approximately 0.5 ml of blood was collected using a Pasteur pipette. This procedure decreased the hematocrit level to between 27 and 30%, but had no effect on the numbers of circulating neutrophils and platelets. On the other hand, mice were rendered plethoric by the method of Jacobson et al.[10]. Briefly, mice received within 6 days prior to irradiation 4 injections i.v. of 0.5 ml of packed washed red blood cells to a hematocrit level between 65 and 70%. Again, this procedure did not affect the numbers of circulating neutrophils and platelets.



The effect of bleeding and hypertransfusion on the absolute numbers of 5-day endogenous spleen colonies of given morphological type. While bars, irradiated-only animals; black bars, irradiated and bled animals; spotted bars, hypertransfused and irradiated animals.

Results and discussion. Total numbers of macroscopically and microscopically visible colonies in 2 separate experiments utilizing either WB/Re or AKR mice to rule out the effect of specific genetic background are shown in the table. Postirradiation bleeding increased the total number of colonies more than 2 times, while hypertransfusion completely prevented formation of macroscopic colonies and produced a 5–10-fold decrease in the number of microscopically visible colonies. As shown in the figure in irradiated-only and irradiated and bled animals, colonies of all 7 morphological types were observed. In irradiated-only animals the most frequent were pure erythropoietic and granulopoietic colonies accounting together for approximately 75% of colonies. Pure M and various types of mixed colonies constituted together approximately 25%. Postirradiation bleeding not only increased the number of E colonies, but actually had a similarly profound effect on the number of G and mixed colonies. For instance, the total number of mixed colonies of all types per spleen increased following bleeding of WB/Re mice from 4.6 ± 1.1 to 20.2 ± 3.2 and following bleeding of AKR mice from 3.1 ± 0.8 to 16.7 ± 2.1. Therefore, all types of TE-CFU hemopoiesis were stimulated by bleeding and not erythropoiesis only. On the other hand, as shown in figure 1 hypertransfusion inhibited all types of TE-CFU hemopoiesis. In fact, erythropoiesis was completely absent by our morphologic criteria and granulopoiesis was drastically reduced, while megakaryopoiesis appeared to be the least affected, although because of the low frequency of megakaryocytecontaining colonies this was subject to greater error than in the case of E or G colonies. Spleens of hypertransfused animals appeared to be hypocellular and in fact resembled organs of lethally-irradiated mice. Similar results were obtained in 7 other experiments utilizing mice of other genetic backgrounds, and could not be related to the dose of radiation used or alterations in the time-course of the appearance of TE-colonies in hypertransfused animals.

These studies have shown that TE-CFUs are tripotent and are able to give both erythropoietic, granulopoietic and megakaryocytic progeny. Although possessing similar differentiative capacities TE-CFUs in various assays express different properties from CFU-S. First of all, it was shown that the TE-CFUs, in order to differentiate in any direction, pass through the stage sensitive to erythropoiesis modulating factors like bleeding and hypertransfusion. Secondly, as was mentioned, they are able to give differentiated progeny earlier than CFU-S, i.e. within 5 days. Thirdly, they are non-transplantable. Fourthly, various genetic loci[5,8] are

engaged in different ways in their regulation and the genetic defects in TE-CFUs could be separated from the genetic defects in CFU-S[8].
Therefore, according to these results, in the murine hemopoietic system 2 separate classes of tripotent hemopoietic cells operate; CFU-S and TE-CFUs.

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Nonspecific esterase activity in monkey thymus lymphocytes; study of distribution in lymphocyte subpopulation

S. Kato and K. Kurihara

Department of Anatomy, Medical College of Oita, Oita 879-56 (Japan), 29 December 1980

Summary. A subpopulation of monkey thymus lymphocytes was investigated by direct nonspecific esterase staining of E-rosette forming lymphocytes. Thymus lymphocytes had high nonspecific esterase activity and E-rosette formation, but the level of their in vitro lectin responses was very low.

Nonspecific acid α-naphthyl acetate esterase (ANAE) activity, rosette formation with heterogeneic erythrocytes and responsiveness to some mitogens have been evaluated as markers for peripheral T-lymphocytes in laboratory rodents and in man. Whether the ANAE marker, E-receptor and lectin response would also be predominantly expressed by thymus lymphocytes was of considerable interest. Previously, we reported that human thymus lymphocytes had low blastogenic activity to lectin, although they had a high degree of E-rosette formation[1]. It has been reported that percentages of ANAE positive cells in the thymus from human[2,3] and mouse[4] were lower than those in peripheral blood. The present study was undertaken therefore to investigate the relationship among nonspecific esterase activity, cell surface receptor for sheep erythrocytes and in vitro lectin responses in thymus lymphocytes and blood lymphocytes from monkeys.

Materials and methods. Fresh thymuses were obtained from 30 young adult Japanese monkeys *(Macaca fuscata)*. A suspension of thymus lymphocytes was prepared by mincing the tissue into fragments on a 60 stainless steel mesh in chilled Hanks balanced salts solution. Blood lymphocytes were also prepared from heparinized venous blood from the animals from which the thymuses were obtained, using the Ficoll-Isopaque (Lymphoprep, Nyeggard & Co. As., Oslo) density gradient method for human lymphocytes, as previously described[5].
Spontaneous rosette formation between lymphocytes and sheep erythrocytes was assayed by the method reported in detail elsewhere[1]. Briefly, equal volumes (0.1 ml) of lymphocyte suspension (5×10^6/ml) and sheep erythrocytes (2×10^8/ml) were mixed in short test tubes. These mixtures were incubated at 37 °C for 10 min, then centrifuged at 700 rpm for 5 min. After 16 h incubation at 4 °C, the pellets

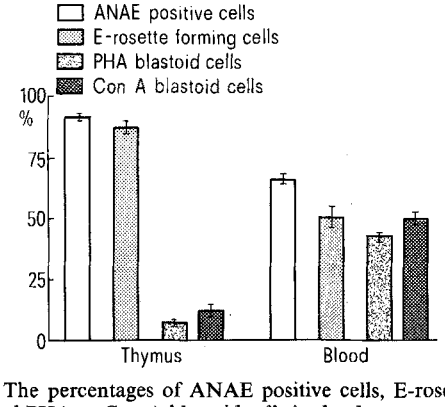

Fig. 1. The percentages of ANAE positive cells, E-rosette forming cells and PHA or Con A blastoid cells in the thymus and peripheral blood from monkey.

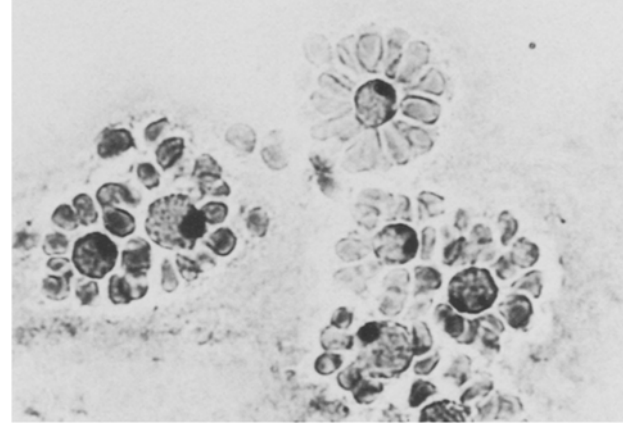

Fig. 2. ANAE positive and E-rosette forming lymphocytes from monkey thymus, × 700.