Lectin-induced lymphocyte stimulation in pigs – a kinetic study of NORs

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Summary. Early reactions of rRNA-cistrons in lectin-stimulated PBL cultures of domestic pigs are shown. Interphases showing 3 or more clusters of stained areas were attributed mainly to cells after first division. Clear differences are shown between PHA- and ConA-induced NOR-expression patterns compared to the patterns in PW-stimulated cultures.

Lectins are sugar binding and cell-agglutinating proteins, widely distributed in nature. Lectin stimulation of lymphocytes causes a characteristic series of biochemical events that lead to blast transformation. Very early events include changes in permeability[2] of membranes, acetylation[3] and phosphorylation[4] of histones and elevation of intracellular cyclic nucleotide content[5]. Later, RNA[6] and protein[7] synthesis accelerate and the activity of several enzymes is increased[8]. At about 48 h, active DNA-synthesis occurs and later the cells enter mitosis. In man the position of the ribosomal cistrons coding for 18S+28S RNA on the secondary constrictions of the short arms of the 5 acrocentric chromosomes was originally determined by in situ hybridisation studies[9,10] and these nucleolar organizer regions (NORs) have been confirmed in many cases with the use of silver staining techniques[11,12]. In the domestic pig the main sites of NORs are the secondary constrictions of chromosomes 8 and 10[13] following Reading nomenclature[14]. In these animals about 1% of peripheral blood lymphocytes (PBL), both sheep red-cell rosette forming and non-rosette forming cells, have been shown by means of DNA-synthesis to be cycling[15]. Pig lymphocytes are known to be activated by phythemagglutinin (PHA), phorbol myristate acetate and soya bean agglutinin (SBA), but not by wheat germ agglutinin (WGA)[16,17]. Different lectins are thought to bind with different affinities to the carbohydrate moiety of the particular glycoprotein which mediates transformation in certain T- and/or B-cell subsets.

The aim of the present paper was to demonstrate the progression of nucleolar activity after stimulation of lymphocytes by nonspecific lectinchallenge and to compare it with the changes occurring in NORs, as revealed by silver-staining, these being a suitable early sign of lymphocyte activation in distinct lymphocyte-sets responsive to 3 different lectins (PHA, PW and ConA).

Materials and methods. Short-term blood cultures from 3 male pigs were grown in 8 ml Parker-medium 199 supplemented with 10% foetal calf serum (FCS). As lectins 0.2 ml PHA (Wellcome Research Lab., Beckenham, Engld. BR3, 3BS), PW (Fa. Seromed Kat. No. 5060 70802) and ConA (Fa. Seromed Kat. No. 3050) were added. The cultures were harvested after 4, 8, 12 and 24 h and prepared as described by Moorhead[18]. Silver staining was done after Goodpasture and Bloom[11]. About 2000 cells (total from all 3 pigs) were counted for each time category. Adequate control cultures, i.e., cultures under identical conditions, but without the above mentioned lectins, were set up for the same pigs.

Results. The progression of nucleolar activity after treatment of lymphocytes with PHA, PW and ConA is seen in figure 1. Figures 2 and 3 depict silver staining of lymphocytes. After only 8 h exposure to the lectin an increase in silver staining was observed, which was characterized by the formation of typical clusters (see fig. 1, cat. II and III, and fig. 2a, b, i–n). Above all, this increase was due to category III cells. A characteristic increment of cells of category IV was not observed before 48 h culture. This pattern of increment of cells was more pronounced in PHA- than in PW- or ConA-activated cultures. In the control cultures no detectable increase of silver staining was observed. In the 48 h lectin-stimulated cultures metaphase plates were also examined for the presence of differentially stained regions. The secondary constriction of 1, 2, 3 or 4 chromosomes (chromosomes no. 10 and no. 8, following the Reading nomenclature[14] appeared as heavily stained NORs.

The table shows the distribution of stained NORs in the metaphase of PHA-, PW- and ConA-stimulated cultures.

By means of the χ^2-test we examined whether there was a significant difference in the response of the cell cultures to the 3 lectins. The distribution of stained NORs in the PW-stimulated cells differed significantly from that in the others ($p < 0.01$). There were no significant differences between PHA- and ConA-stimulated cells.

Lectins	No. of NORS in methaphasis				Total No. of meta-phases
	1 NOR	2 NORs	3 NORs	4 NORs	
PHA	19	94	22	1	136
PW	3	100	17	3	123
ConA	8	48	3	1	60

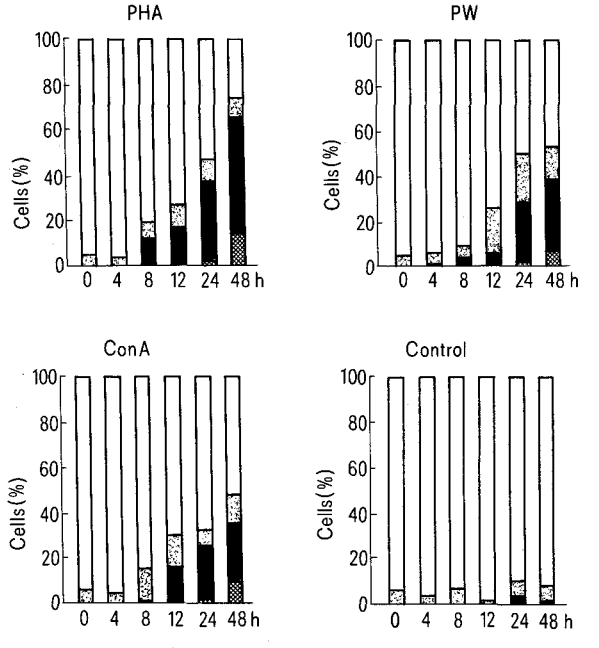

Figure 1. Distribution of the various interphase-cells classified in 4 categories in course of stimulation with different lectins.
□, Category I: cells with 1 or 2 stained areas.
▨, Category II: cells with 3 or more stained areas.
■, Category III: cells with 1 or 2 clusters of stained areas.
▩, Category IV: cells with 3 or more clusters of stained areas.

Discussion. Our results of a strong stimulation mediated by 3 lectins for pig PBL are in accordance with data in the literature[19]. The interphase NOR-expression patterns in our studies indicate that PHA was the most potent stimulator of the 3 lectins. Studies on the kinetics of division in PHA-stimulated pig lymphocytes[20] indicated that in the pig, at a harvesting time of 48 h, 78% of mitoses are 1st mitoses, 19% are 2nd and 3% are 3rd mitoses. In contrast, in PHA-stimulated human lymphocytes at 48 h practically all the dividing cells are 1st mitoses products. Lezana et al.[20] concluded that pig lymphocytes are able to divide earlier and more frequently than human lymphocytes. At a

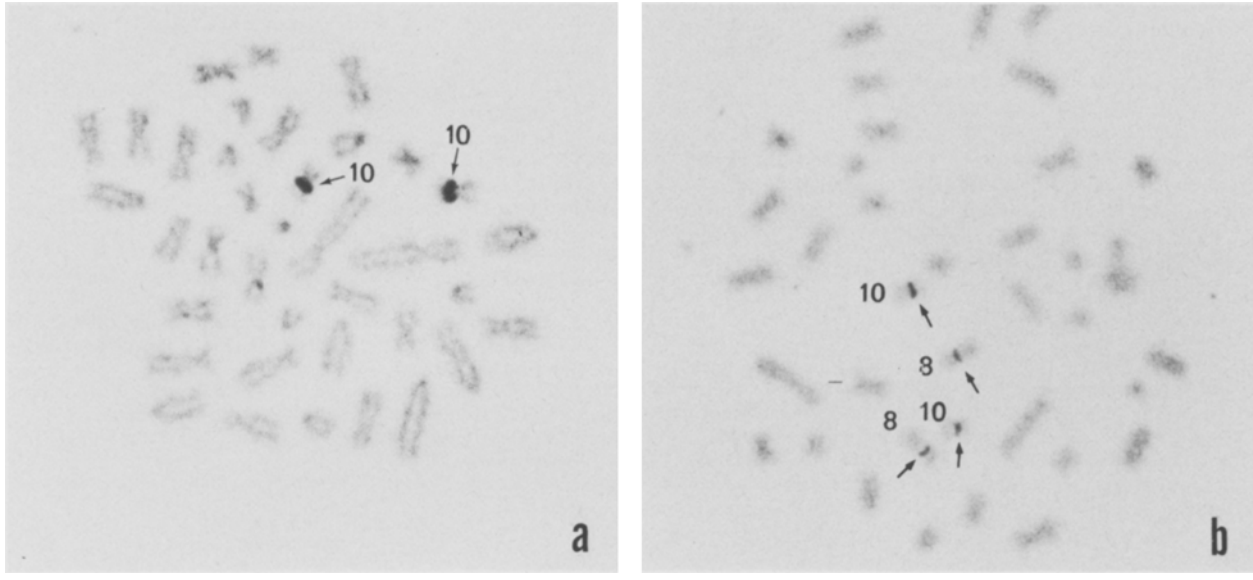

Figure 2. Silverstained NORs in metaphase, *a* cell with 2 NORs, *b* cell with 4 NORs.

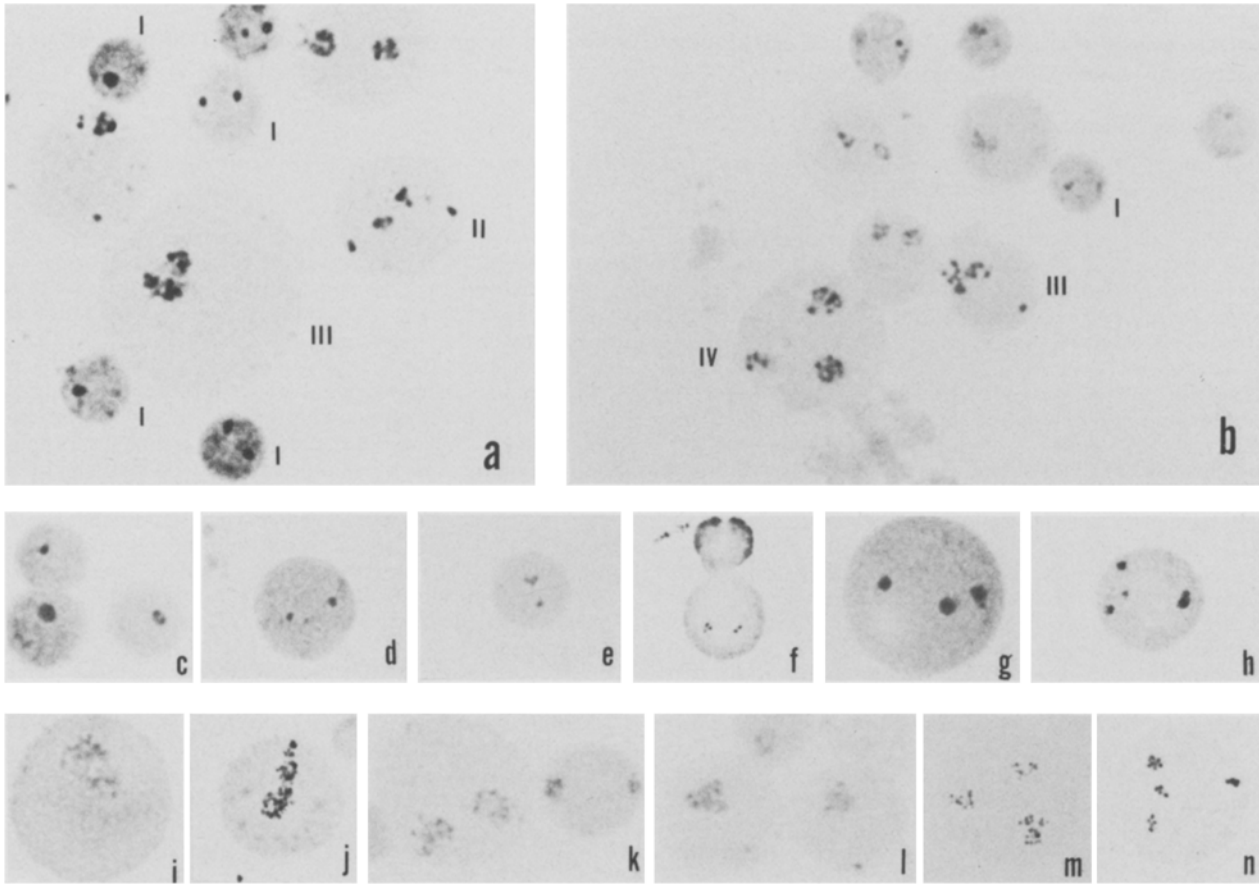

Figure 3. Silverstained NORs in interphase, *a, b* group of stimulated cells of different categories, *c–f* category I, *g–h* category II, *i–l* category III, *m, n* category IV.

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

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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-