tibodies suggesting that there was an abrogation of self-tolerance maintained by the thymus or thymus-derived cells. Neonatal thymectomy alone has also been shown to induce or enhance autoimmunity in several systems[12-14]. This suggests that irradiation does not play a direct tissue-damaging role in the genesis of the lesions. This aspect has been extensively studied in a rat model of autoimmune thyroiditis in this laboratory, where it has been shown conclusively by shielding[6], lymphoid cell transfer[15] and also post-irradiation thyroid grafting[16] studies that irradiation does not directly cause or even potentiate the lesions observed. Furthermore, only the combination of early thymectomy and irradiation led to the development of severe lesions whilst either thymectomy or irradiation alone resulted in little if any change[6]. These findings also suggest that some of the consequences of severe irradiation previously ascribed to a direct damaging effect may be generated via autoimmune mechanisms.

Although the influence of genetic background on the susceptibility to autoimmune changes is clearly evident from the strain differences it was not possible to relate this to the major histocompatibility complex. It is likely that other non-MHC linked genes may be important in influencing the susceptibility in this particular situation. However, the H-2 basis of susceptibility to autoimmune diseases in this particular context needs further examination in other strains of mice with similar or differing H-2 haplotypes.

This approach should have many applications in studying basic autoimmune pathogenesis. For instance, it affords an opportunity of investigating the mechanisms responsible for a wide range of autoimmune phenomena, particularly those involving the thyroid, stomach and haemopoetic system. Furthermore, the availability of a wide variety of inbred congenic and mutant mouse strains will enable the relationship between the genetic make-up and a particular expression of the spectrum of autoimmunity to be examined in depth.

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The silver-staining technique – a tool for characterizing lymphocyte populations in mammalian peripheral blood

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Summary. Nucleolus organizer regions (NORs) of the peripheral blood lymphocytes (PBL) of 9 mammalian species were analyzed by means of a silver-staining procedure. Species-specific NOR patterns were demonstrated. The number of NOR chromosomes was positively correlated with the nucleolar coefficient, and negatively correlated with the relative frequencies of uninucleolar cells in PBL interphase.

Silver-staining methods have been applied for the localization of nucleolus organizer regions (NORs) in metaphase and/or in interphase cells[2-5]. The classical works of Heitz[6] and McClintock[7] on plant material demonstrated that the sites of nucleolus formation at telophase occur at the NORs present on certain chromosomes. Heneen[4] confirmed, on mammalian monolayers, that the silver-staining observed at metaphase corresponds to that expressed at later stages of mitosis and during early interphase, and is nucleolar in nature. Beran and Pospichil[8], using toluidine blue staining, found species differing in their ratio of uninucleolar and multinucleolar lymphocytes and in the nucleolar coefficient (mean of silver-stained nucleoli per lymphocyte) in peripheral blood lymphocytes (PBL). PBL are highly heterogeneous, and most of them are believed to be highly differentiated (G_O-phase) and to divide seldom. However, most of them can be activated by both unspecific and specific antigens to re-enter the cell cycle and become cycling. In human PBL, an increase in silver-staining from 1 or 2 stained area(s) to several – up to 5 or more – individual, small areas has been reported after PHA stimulation[5].

The present investigation was aimed at determining the suitability of the silver-staining technique applied for detecting NOR variabilities in unstimulated PBL interphases, by assessing the relative frequencies of PBL with distinct silver-stained areas, and the nucleolar coefficient, and comparing them with the diploid number of NORs, in several mammalian species.

Material and methods. Peripheral blood was taken from unimmunized, healthy, adult individuals, 2 male and 2 female of each of the following species; man *(Homo sapiens)*, rabbit *(Oryctolagus cuniculus)*, horse *(Equus caballus)*, pig *(Sus scrofa)*, dog *(Canis familiaris)*, cat *(Felis catus)*, cattle *(Bos taurus)*, sheep *(Ovis aries)*, and goat *(Capra hircus)*. 10 ml of peripheral blood was centrifuged at $400 \times g$, and the buffy coat withdrawn, fixed with 3:1 methanol acetic acid for 10 min, then pipetted onto slides and air-cried. In later experiments a short, hypotonic treatment (37 °C, 5 min) of the buffy coat cells before fixation seemed further to improve the contrast of the deeply-stained nucleolar chromocenters against pale background structures that sometimes occurred in addition to them.

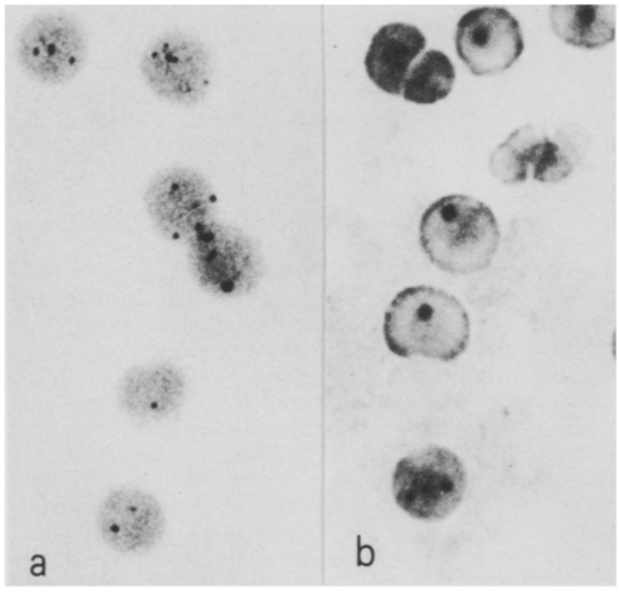
Relative frequencies of PBL with different numbers of silver-stained areas NOR chromosomes per diploid genome, in the different species

Species	Relative frequencies of PBL with different numbers of silver-stained areas					Nucleolar coefficient	NOR chromosomes per diploid genome
	1	2	3	4	5 or more		
Man	0.816	0.117	0.048	0.016	0.030	1.4	10
Cat	0.724	0.237	0.031	0.008	0.0	1.3	4
Dog	0.556	0.200	0.117	0.075	0.051	1.9	8
Rabbit	0.357	0.324	0.179	0.091	0.049	2.2	8
Horse	0.433	0.267	0.206	0.076	0.018	2.0	6
Pig	0.503	0.349	0.136	0.012	0.0	1.7	4
Cattle	0.147	0.327	0.285	0.161	0.080	2.7	10
Goat	0.209	0.281	0.217	0.160	0.127	2.7	10
Sheep	0.193	0.443	0.203	0.077	0.082	2.4	10

Silver-staining was performed as in earlier experiments, the only difference being in the omission of the G-banding step. 1000 cells per individual were evaluated. The significance of the data obtained was tested with the Spearman[10] rank-correlation.

Results and discussion. Our studies show that there is a significant positive correlation ($r_s = 0.62$; $p < 0.05$) between the number of NORs and the nucleolar coefficient; species with high NOR numbers generally showed a higher nucleolar coefficient. There is one clear exception to this; For man, with 10 NORs, a rather small nucleolar coefficient of 1.4 was established. Contrary to this generally positive correlation, NOR numbers correlated negatively with the relative frequencies of uninucleolar cells ($r_s = -0.66$; $p < 0.05$); species with a high NOR number showed a low frequency of uninucleolar cells. Here again, man clearly differed from the other species investigated. It is further remarkable that the number of NORs correlates negatively only with the relative frequency of nucleolar cells; for the other cell types (with 2-5, or more, silver-stained areas) the number of NORs correlates positively. These data plainly show up existing exceptions in the correspondence between NOR number per diploid genome, and silver-stained areas in the interphase. It can further be assumed that uninucleolar cells play a distinct role among the lymphocyte population in peripheral blood.

The interspecific differences probably reflect differences in NOR activities and/or NOR association/dissociation patterns. Anastossova[11] demonstrated on mammalian cell cultures the kinetics of nucleoli within the cell cycle, starting with the appearance of the primary nucleoli in telophase and, the gradual association into a single interphase nucleolus, followed by various stages of dissociation, until their disappearance at metaphase. On the other hand, some recent investigations have given results which led to the assumption that NORs are not associated in unstimulated lymphocytes[12]. It should also be considered, even though most PBL are commonly believed to be blocked 'resting G_0' cells, that the differences between species could reflect species-specific heterogeneity in PBL cell cycle progress until some earlier, or later, stage of gap-phase; however, only a few cells reach S-phase. In the light of these possibilities the concept of 'resting G_0' cells in mammalian peripheral blood lymphocytes should be reconsidered carefully. On the other hand, bi- and polynucleolated human PBL have been reported to represent mainly B-cells while PBL with small single nucleoli were attributable to T-cells[13]. In humans an increase of the nucleolar coefficient of PBL has been reported in certain stages of cancer[14,15] and in allergic diseases[16]. The authors of these papers used toluidine blue staining to depict NORs. The silver-staining procedure applied in our studies allows a simple and reliable demonstration of nucleolus organizer material in unstimulated mammalian PBL. It provides a promising approach to a further definition of mammalian PBL by nucleolar characteristics, and will be helpful in defining functional subpopulations of these cells.



Typically silver-stained PBL-preparations. × 1850. *a* Cattle, *b* man.

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