ANALYSIS OF PATTERN OF rRNA-SYNTHESIS IN LYMPHOCYTES BY THE AID OF A MOUSE-MODELL WITH CELLULAR IMMUNEREACTION. (cytological marker).

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INTRODUCTION:

Many investigations exist on the searching of markers to distinguish lymphocyte populations to enlarge knowledge about immunereaction and also of appropriate markers for breeding programms. The most investigations deal with lymphocyte surface markers. The pattern of rRNA-synthesis in the nucleus visible with silverstaining technique could be a good completion to the other markers.

In this investigation an analysis of this pattern to distinguish between functional or cellcycle pattern and genetical fixed structures was done by the aid of a modell. In this standardized mouse infection modell, infected with Listeria monocytogenes, it is possible to read off the immune status of the mice at any time. Thus conclusions could be drawn concerning the functional status of the cells respectively of the rRNA-pattern in the nuclei.

MATERIAL and METHOD:

180 mice of B6D2F1 were infected with $1X10^4$ organism of Listeria monocytogenes each. It is a standardized dosis inthe modell of Mackaness (1975). During the investigation spleen and liver were tested on germs of Listeria monoo. (fig.1) In 10 days the mice had surmounted the infection.

On day 1,3,4 and 5: 30 mice were cutted each to get the blood for gathering lymphocytes with the method of Bojum (1968). Before droppingpreparation from the fixed lymphocytes were made they were exposed to a hypoton medium for 19 minutes to make plasma bursting. Then the slides were stained with the silver-staining method (Howell and Black, 1980) and analysed microscopical.

The significance of the data obtained was tested with -test.

RESULTS:

The traced kinetics of several forms of nuclei (1kDP, 1m1k, 3k, 1g,2g, Gr) are shown in fig.1 Conspicious is the increase in frequency of the most cells

from day 1 to day 3 and the following decrease from day 3 to day 4.
The forms with small nucleoli (1k - 5k) have thus far a peculiarity that they fall down from a higher level in healthy individuals to a lower level on the first day after infection and increase after this, like the most other cell forms. These other cell forms increase from a lower level in the healthy individuals. The granulated nucleus froms have a different kinetics. Their maximum is one day later on the fourth day.
The nucleus forms with one or two big silverspots (1g,2g) show a different kinetics too. It is opposite to the kinetics of the most forms. It increases till day 1, decreases till day 3 and increases again.
The conspicous difference in the distribution of the frequency of the nucleus forms from the impaired individuals to the healthy one is the great increase in nucleus forms with one small double point (1kDP).

DISCUSSION:

Looking in this modell at the immune status of the animals it can be seen that at day 2 there is the maximum of germs founded. After day 2 the organism is able to eliminate the infectious germs and the curve (fig.1) falls down. The fact that the kinetics of the most cell forms shows a maximum that comes later than the maximum of germ infection is typical for regulatory systems. It can be argued that these cells are in proliferation, since proliferation is the first reaction of the immune system after the interaction with the antigen.
The nucleus forms with 1kDP, which show a conspicious increase at this time must be allied to cells in proliferation. Morphologically the small double point can be explained like this: At the late prophase or early metaphase, when chromosomes are in condensation already, the homolog chromatides are separated and the silverstained NOR-regions on these chromatides can be seen as small double point.
An other nucleus form can be interpreted functionally by the aid of this modell. The increased occurence of the granulated nucleus forms after the proliferation forms can be connected with the lymphokin production, a reaction of the immune system that follows after proliferation.
The morphology of this nucleus form seems to indicate this interpretation too. The lymphokine to stimulate macrophages is probably produced from sensiblizied lymphocytes in this modell.

SUMMARY:

Mice were infected with Listeria monocytogenes with a dosis standardized in this modell.

The kinetics of several nucleus forms were investigated and brought in connection with the immune status of the animals red off from the curve of infected germs of this modell.

Thus the function of two nucleus forms could be estimated.

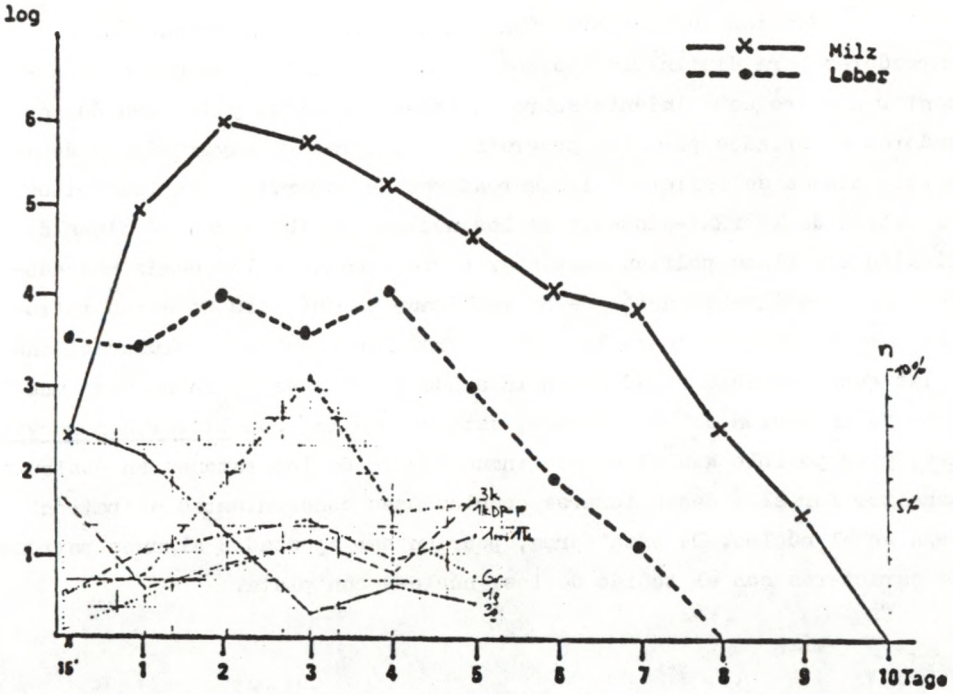

Figure 1:

Kinetics of Listeria monocytogenes in spleen and liver and the kinetics of 5 different nucleus forms.

SUMMARY

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In this way some characteristic pattern in the silverstaining of nuclei could be adjusted.

R E S U M E N

Existen muchas investigaciones sobre la determinación de marcadores para distinguir las poblaciones de linfocitos a fin de aumentar nuestro conocimiento sobre la inmunoreacción, y tambien de marcadores apropiados para los programas de mejora. El mayor número de investigaciones se refiere a los marcadores de superficie en leucocitos. El patrón de la rRNA-síntesis en los núcleos visibles con técnicas de tinción con plata podrian completar perfectamente a los demás marcadores. En esta investigación se ha realizado un análisis de estos patrones para distinguir entre las estructuras funcionales ó celulocíclicas y las genéticamente fijadas con la ayuda de un modelo. En nuestro modelo tipo de infección de los ratones, esta se realizó con Listeria monocytogenes, y es posible léer el estado inmunológico de los ratones en cualquier momento. Por ello deben tomarse conclusiones concernientes al patrón rRNA en el núcleo. De esta forma, podrian ser ajustados algunos patrónes de caracteres con el teñido de los núcleos con plata.