

No 12: NEWS from TECNOMOUSE



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Humoral immune response in-vitro

One of the greatest challenges facing Susi and Adi in their daily routine is the production of human antibodies, important for diagnostic procedures and the therapy of severe illnesses.

The methods used up to now are somewhat tedious and time-consuming and the yields are less than satisfactory. This can be explained by the low statistical probability of a clone growing as a result of the fusion of peripheral blood lymphocytes with myeloma cells and in turn producing the desired antibodies. An in-vivo immunisation procedure successfully carried out using animal cells cannot be used for humans for ethical reasons.

Susi is well aware of the problems that arise in the formation of human antibodies from many personal discussions with her colleagues and with her laboratory head. She still gets excited when she thinks of the experiments carried out in this area: The laboratory head, in an experiment in TECNOMOUSE, succeeded in carrying out important steps in the stimulation of human immune defence. He was able to:

- Achieve antigen acceptance in-vitro
- Achieve presentation on the outer cell membrane
- Achieve stimulation of T-lymphocytes

Susi always gets excited when she reports on what actually happened during this experiment:

The mononuclear cells (MNC) were separated from 400 ml of patient blood. 2×10^8 MNC were then inoculated into the culture cassette. Subsequent to addition of the corresponding antigen, a sample was removed from the extra-capillary space of the cassette for FACS analysis (fluorescence-activated cell sorter). This analysis showed that 80% of the cells were lymphocytes and 20% monocytes. The differentiation marker of the lymphocytes and the functional markers of the monocytes and lymphocytes were measured in order to obtain an overview of possible differentiation or stimulation.

During the 34-four-day cultivation phase, the cells were supplied with medium using the recirculation mode. In this mode, the important low molecular substances remain available for the cells and are not removed with waste. The cells also require no foetal calf serum as additional feed; rather, they receive human serum albumin and transferrin.

"I remember quite clearly the harvest after 34 days", says Susi. "Gentle but thorough; macrophage types like you would have become terribly excited". Adi was not impressed and explained the results of the FACS analysis: After 34 days, 17% of the cells are still lymphocytes and 83% monocytes. The proportion of lymphocytes (T-helper cells) increased from 44% on the first day to 63% on day 34. Memory cells increased from 19% to 72% and the antigen-presenting cells increased from 10% to 20% of the cell population.

The vitality of the cell population as a whole was 90% compared with the approximately 4% of a parallel suspension culture that was being analysed.

As far as Susi was concerned this meant that the monocytes felt really at home in the TECNOMOUSE. They not only consumed the antigen hungrily but digested it in such a way that it appeared as a small peptide on its surface. "This is called antigen presentation", said Adi. "This in turn is also extremely important for activating lymphocytes as these can not cope with the antigen on its own."

The laboratory head explained to both of them the results of the successful experiment from his point of view. Of the 5 phases of the humoral immune response:

1. Activation of the macrophages as phagocytes or antigen-presenting cells.
2. Activation of the T-helper cells as "teachers" of the B-lymphocytes.
3. Activation of B-lymphocytes themselves.
4. Proliferation and differentiation of activated B-cells to plasma cells.
5. Synthesis and secretion of antibodies via plasma cells.

Thus, the first two of these 5 have been successfully carried out under in-vitro conditions in TECNOMOUSE.

We can show that the macrophages consume the antigen and then make themselves available to the other cells. We also find activated T-helper cells.

The laboratory head is convinced that he can complete other steps under in vitro conditions in further experiments

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