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In reply please refer to : BS
Prière de rappeler la référence :

19 August 1960

Dear Dr Szilard,

When visiting Copenhagen last month Dr Ole Maaløe showed me your papers on the Control of the Formation of Specific Proteins and On the Molecular Basis of Antibody Formation.

I now intend to study these papers a little more closely. Meanwhile, I take the liberty of sending you a preprint of my manuscript on Immunological Theory which will appear in this year's Annual Review of Microbiology. Though my approach to the question of antibody formation is quite different from yours, I hope that my paper will interest you.

I saw a glimpse of you once when you paid a lightning visit to the California Institute of Technology, Pasedena, in 1954. I hear that you are in hospital and send you my best wishes for your recovery.

Yours sincerely,

N K Jerne

N. K. Jerne

Dr Leo Szilard
Enrico Fermi Institute for Nuclear
Studies
University of Chicago
Chicago
Illinois
USA

June 7, 1963

Professor Niels Jerne
Department of Microbiology
The Medical School
University of Pittsburgh
Pittsburgh, Pennsylvania

Dear Dr. Jerne:

When I recently saw you in Washington, you said that you had no plans of applying your new method for demonstrating the production of antibodies by single lymphatic cells to the problem of the simultaneous production of two or more antibodies by the same cell.

The experiment to which I am referring consists in immunizing a rabbit with the red cells of two different species and then plating lymphatic cells of the immunized rabbit on agar which contains a mixture of the two kinds of red cells that were used to immunize the rabbit.

Yesterday, I discussed this experiment with Dr. Arthur Silverstein of the Armed Forces Institute of Pathology, Walter Reed Hospital, Washington 25, D.C., and we thought we might try to do this experiment, unless you have in the meantime changed your mind and would want to do the experiment yourself. I told Dr. Silverstein that I would write you to get your reaction.

I am off to-day to attend a meeting in Cold Spring Harbor, and soon after my return to Washington, I take off again to go to Geneva. I do not as yet know just when I would return to Washington from Europe.

If you think that we ought to go forward with this experiment, then in the meantime perhaps Dr. Silverstein could visit you in Pittsburgh to learn whatever he would need to know about your method. I am sending him a copy of this letter and perhaps you might want to write him directly, with a carbon copy of your letter sent to me at my Washington address. While I am in Europe, my Washington mail will be forwarded to me.

With kind regards,

Sincerely,

Leo Szilard

UNIVERSITY OF PITTSBURGH
SCHOOL OF MEDICINE
PITTSBURGH 13, PENNSYLVANIA

DEPARTMENT OF MICROBIOLOGY

June 14, 1963

Dr. Leo Szilard
Hotel Dupont Plaza
Dupont Circle and New Hampshire Avenue N.W.
Washington 6, D. C.

Dear Doctor Szilard:

Thank you very much for your letter concerning the problem of the simultaneous production of more than one antibody by the same cell. After having seen you in Washington last month, we have done a few experiments in this direction, ~~namely~~ mainly in order not to feel too much discouraged by repeated negative results from in vitro stimulation experiments.

We immunized mice with an injection of sheep red cells. The 4th day spleen from these mice gave many plaques on agar plates containing sheep red cells and no plaques on plates containing rabbit red cells. We immunized other mice with rabbit red cells. The 4th day spleens from these mice gave many plaques on agar plates containing rabbit red cells and no plaques on plates containing sheep red cells. A third group of mice received an injection of a mixture of rabbit and sheep red cells. The 4th day spleens of these mice gave many plaques both on agar plates that contained sheep red cells only and on plates that contained rabbit red cells only. All agar plates so far mentioned contained 400 million red cells in the 2 ml top layer. On plates containing a mixture of 400 million sheep red cells and 400 million rabbit red cells, the spleens from the 3rd group of mice gave a number of plaques that was approximately equal to the sum of the numbers observed on the plates containing only one type of red cells. Unfortunately, these plaques were quite distinct though a little hazy. We saw none that were entirely clear and that could be identified as a plaque resulting from a double producing cell. Many of the plaques, however, are quite small as you will recall from my paper stating the variation in plaque size. It appeared quite difficult even if a small plaque had looked clear to obtain confidence that such a plaque would represent a double producer.

The difficulty here is in the presumed rareness of the phenomenon. The more rare a plaque type is the more characteristic it must be in order not to be wiped away as an accidental oddity.

It is possible that some cells could produce mainly one antibody and only little of another antibody. In that case, one might expect a plaque that was clear towards the center and more hazy in the periphery. We felt, however, that such differences could not be picked up under our present experimental circumstances. What we would

need is an experimental situation in which only double producers would produce a plaque or at least in which there would be a marked difference between a double producer and a single producer. We have tried the other day to use plates containing 10 X as many red cells, namely 4×10^9. The plaques on such plates are quite distinct, though very tiny like stars in the heaven. We now plan to try such plates in the experiment for double producers but I doubt whether much reliance can be placed on any results from this approach.

Maybe the problem needs quite a different procedure. I could imagine, for example, that a plaque technique could be evolved utilizing a ~~the~~ hemolytic toxin that is antigenic and cells from a mouse immunized with this hemolytic toxin. If we incorporated the toxin in the bottom layer of the plate and added a top layer containing red cells and spleen cells from the immunized mouse, the toxin from the bottom layer would diffuse up and lyze all red cells in the top layer but perhaps the red cells surrounding anti-hemolysin producing spleen cells might be protected so that red plaques would result consisting of clusters of unlyzed cells. If this were feasible, then in a mouse immunized with red cells and simultaneously with the hemolytic toxin, double producing cells might arise that would secrete ~~anti~~ hemolysin as well as anti-hemolytic antibody. On a hemolysin containing agar plate such cells would produce plaques of unlyzed red cells as described before. Upon the addition of complement, however, these red cells would be lyzed because of the hemolytic antibody and thus double producers would be represented by plaques that appeared in the first step but disappeared upon the addition of complement. Other systems of such a nature could be evolved that might give more clear results than our present approach.

I hope this messy description is understandable

If Dr. Arthur Silverstein is still interested in this type of experiment, we would be very pleased to show him our techniques and results here in Pittsburgh whenever a visit would be convenient to him. We have no objection against Dr. Silverstein trying to obtain more conclusive data concerning this tantalizing problem.

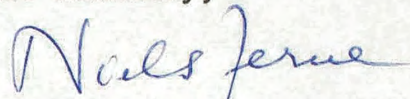
At present, however, I wonder whether much progress can be expected from efforts in this direction. Double producers, as you know, have been shown in the experiments of Attardi and Lennox and also in a very small proportion by Nossal. All these experiments were done in hyper-immune animals. One might expect that double producers after primary stimulus are very few if at all present. One might also expect that double producers would arise more readily if the two antigenic determinants considered were on the same particle and not on two different particles such as two red cells. Experiments could no doubt be devised with protein molecules of different types exhibiting cross reacting antigenic determinants. I expect cells that produce antibody originate from multi potential cells by differentiation which may involve a number of cell divisions. It may be a rare occurrence that two potentialities are expressed simultaneously. Immunology is still in such a primitive state that, for example, we don't even know whether

the 100,000 spleen cells that are secreting antibody on the 4th day after one antigenic stimulus have arisen by multiplication or not. Nor do we know whether antigen has ever entered these cells or even come into contact with these cells - or whether the stimulus has arisen in phagocytic monocytes or leucocytes that have taken up the antigen initially.

There are many problems of a fundamental nature which I have thought of for quite some time in this field and I should be very glad if I could have an occasion to discuss these with you. I shall be going to a conference of the Pan American Health Association during the whole of next week. If you should be away in Geneva during that week, I might visit you later.

With best wishes and kindest regards,

Yours sincerely,



Niels K. Jerne, M. D.
Professor of Microbiology and
Chairman of the Department

NKJ/br