February 21, 1957

Dr. David W. Talmage
Department of Medicine, BH M 564
The University of Chicago
Chicago 37, Illinois

Dear Talmage,

I do not know whether I shall see you before I leave tomorrow for Denver. Therefore, I am sending you this memo. If you get it in time and after you have thought it over, perhaps we can still talk about it over the telephone.

It seems to me that your theory suggests a simple experiment which is as follows:

Immunize ten rabbits with an antigen A, and after antibody appears (primary response) pool the serum of these rabbits, and place one-half in container 1, and the other half in container 2. Subdivide container 1 into aliquots a, b, c, d, e, etc. Add to aliquot a a small amount of antigen A, and to each subsequent aliquot increasing - but still small - amounts of antigen A. The purpose of this is to absorb the most avid antibody from these aliquots. Subsequently, remove the antigen from each aliquot. If red cells of another species are used as the antigen, they can be removed by repeated centrifugation. In the following I shall refer to the aliquots so treated as depleted aliquots because they are depleted in the most avidly combining antibody.

We now add to each such depleted aliquot the same amount of antigen A and inject ~~the~~ each such mixture into a ~~rabbit~~ different rabbit. We should expect that ~~this~~ these rabbits will respond by producing at least some avidly combining antibody. In contrast to this, the control rabbit - into which we inject a comparable amount of non-depleted antibody taken from container 2 mixed with the same quantity of antigen A - should not respond by producing a comparable amlunt of avidly combining antibody.

Sincerely,

Leo Szilard

m

February 25, 1957

Dr. David Talmadge
Department of Medicine
Billings Hospital
Chicago 37, Illinois

Dear Talmadge:

The following would be, I believe, a great improvement over the experiment about which I wrote you just before leaving Chicago, and which we discussed over the telephone.

Take 2 antigens, A and B -- for instance, bovine serum albumin and some other serum albumin -- so selected that antibody a, made against antigen A, shall cross-react with antigen B. The experiment, then, is as follows: We immunize 10 rabbits with antigen A, pool the serum, and deplete the serum by absorbing ~~out the~~ it out with antigen B, and then removing from the mixture the antigen B. The depleted serum thus obtained now contains only ~~that~~ such fraction of the initially present antibody a which does not react with ~~the~~ those antigenic configurations ~~which~~ that antigens A and B have in common.

We now take an experimental rabbit and a control rabbit, neither of which have been previously injected with anything.

Into the experimental rabbit we inject the depleted antiserum a, and into the control rabbit we inject the crude antiserum a. We also inject simultaneously into both rabbits, ~~is~~ a not too large dose of antigen A.

The antibodies formed in the primary responses by these 2 rabbits must then be examined. ~~A~~according to your theory, we shall expect that the experimental rabbit should form antibodies that will react both with antigen A and antigen B. Further, ~~The~~ the antibodies formed by the experimental rabbit, after depletion with antigen B, should not be able to react with antigen A. The control rabbit should, of course, not show any antibody formation against either A or B.

Now, I believe that the above described experiment should enable us to distinguish -- according to whether it comes out the way your theory predicts, or contrary-wise -- whether the failure of the control rabbit to produce antibody is due to the ~~renewal~~ removal of the injected antigen " " by the ~~previously~~ passively transferred immune serum, or whether, as your theory predicts, the lowering of the titer of a certain specific antibody by the antigen is the responsible circumstance.

A variant of the experiment described above would be as follows: The experimental rabbit and the control rabbit are both first immunized with antigen A, and then, just before evoking the anamnestic response by a second injection of antigen A, the experimental rabbit is given an injection of "depleted" antiserum a, and the control rabbit is given an injection of the crude antiserum a.

Sincerely,

Leo Szilard

LS:hw

April 15, 1957

Dr. David W. Talmage
Department of Medicine
Billings Hospital M-564
Faculty Exchange

Dear Dr. Talmage:

I wonder if we could do a simple experiment with rabbits here in Chicago which is aimed at discovering whether Erhlich-Jerner's theory is correct, without giving any information concerning your modification of the Jerner theory.

The experiment is as follows: We look for a male rabbit which is a good antibody former against antigen A and a bad antibody former against antigen B, and we look for a female rabbit which is just the opposite; i.e. a bad antibody former against antigen A and a good antibody former against antigen B. These rabbits are then mated and the offspring is tested for antibody formation against antigens A and B.

If Jerner's concept is right, one should expect good antibody formation against an antigen to be inherited as a Mendelian dominant. Preferably both antigens used should be similar in the sense that they should either both be soluble antigens (or with particulate antigens) against which antibody A is formed primarily by the same tissue and the same cell type in the same tissue -- as far as this can be ascertained. Naturally, more than two antibodies and the descendants of more than one rabbit pair should be tested.

It seems to me this is a very simple experiment of a semi-quantitative type, and that the simple basic question which it raises should not be left unanswered irrespective

of its bearing on any specific theory of antibody formation.

I plan to call you soon and perhaps we could have lunch together or perhaps meet after 5:00 for a drink.

Sincerely,

Leo Szilard

m
Encl.

UNIVERSITY OF COLORADO
MEDICAL CENTER
4200 EAST NINTH AVENUE
DENVER 20, COLORADO

February 9, 1960

COLORADO GENERAL HOSPITAL
COLORADO PSYCHOPATHIC HOSPITAL
SCHOOL OF MEDICINE

Dr. Leo Szilard
% Dr. Maurice Fox
Rockefeller Institute for Medical Research
66th Street and York Avenue
New York 21, N.Y.

Dear Leo:

I have just completed an analysis of your paper, THE MOLECULAR BASIS OF ANTIBODY FORMATION. I would like to make certain that I understand correctly your views. Accordingly, I have prepared the enclosed statement in which I have expressed your theory in my own words in a form of six postulates. After each postulate I have discussed briefly its implication to illustrate further my interpretations. I would appreciate your looking this over and letting me know if, where, and how I have misconstrued your meaning.

Best personal regards,

Dave

David W. Talmage, M.D.

DWT:hh

Enclosure

POSTULATES

1. Cells of lymphocytic series first exist in omnipotential state with respect to antibody production. At this time any cell properly stimulated can make any of approximately 10,000 different globulins. Each globulin has a corresponding repressor (REP_j) and coupling enzyme (C_j). Since the concentration of all C_j cannot exceed 10^{-4} molar the individual C_j moiety has a maximum concentration of 10^{-8} M.

2. Antigen coming into omnipotential cell selectively precipitates or absorbs coupling enzyme C_j causing production of globulin, which by further absorbing repressor, locks cell into permanent state of antibody synthesis.

Statement that cell locked for one antigen is no longer sensitive to another is subject to experimental test. Question: Is frequency of double producing cells greater when antigens are chemically bound together on same molecule or particle than when antigens are given separately? Nossal is currently working on this problem with Salmonella antigens. Failure to find that molecular or temporal proximity has any influence on frequency of double producing cells will cast serious doubt on premise that antigen plays a role in the differentiation of antibody producing potential.

Explanation on top of page 11 of increased antigenicity of hapten coupled to antigenic protein is difficult to understand. Why would absorption of other coupling enzymes effect rate of production of repressor in hapten system?

3. To explain anamnestic response, an enzyme is postulated which inhibits cell division and is absorbed by antigen antibody precipitates . A literal interpretation of this statement would prohibit anamnestic responses in non-precipitating systems.

Experimental evidence would indicate that cell division responsible for anamnestic response takes place before second injection of antigen. X-radiation blocks anamnestic response more successfully if given 10-50 days before 2nd injection than 24 hours before. Also, anamnestic response may give 1000 times antibody of primary response within a few days- a time too short to account for multiplication. This postulate can stand or fall by itself without affecting first two.

Explanation of increased antigenicity in secondary response of hapten bound to homologous protein is not unique to this model. Antigen-antibody precipitates are known to be more antigenic than soluble antigens, and particulate antigens in general are 100-1000 times as antigenic as the same antigen in solution. We have increased antigenicity of BSA by precipitating it with RNA and Dixon's group has done the same by absorbing it on charcoal. Simplest explanation is that large particles increase concentration to which individual cells are exposed.

4. If a cell is locked to two antibodies simultaneously, stimulation by one antigen will produce anamnestic response to other. We have looked for this effect with BSA and Forsman antigens, but could not find even a suggestion. Dubert's results can be explained by a cross reaction. I agree that more work should be done here because this, like the question of the frequency of double producers, is critical to the question of the cause of "differentiation" of antibody forming potential.

5. Failure of antibody production in newborn animals is due to high level of repression factors within the cell. This does not explain failure of adult cells to produce antibody in newborns or of production of antibody in adults by cells from newborn.

6. Failure of antibody production at several weeks of age following antigen injection at birth is due to ability of antigen to substitute for repressor in inhibiting antibody formation. It is not clear why this does not happen when antigen is first given later. It is necessary to make an additional postulation that antigen must penetrate into a cell further to produce repression than to produce stimulation. This is similar to Crampton's views presented at the Federation Meetings last year. This model might account for inhibitory effect of haptens given simultaneously with complete antigen.

Nothing is said in this model about development of the plasma cell following antigenic stimulation and the increase in RNA/DNA ratio of spleen and lymph node for only a few days post-antigen. Nor does the postulation of single microsome for each antibody account for the rate of antibody synthesis, approximating 1000 molecules/cell per second. It seems to me that an important repressor involved in these cells must have to do with the rate of ribosome production. Antibody production might be automatic in the presence of the proper ribosome if the rate of ribosome production were controlled. What about the apparent importance of the nucleolus in the plasma cell?

The major difference between this theory and that of Burnet's, Lederberg's and mine is that you postulate a role for antigen in the differentiation of antibody producing potential of cells, whereas we postulate that this differentiation occurs before the injection of antigen. Tolerance is explained in your model by antigen having opposite effects on a cell depending on its location within the cell and in our model depending on the stage of cell maturation. The last point may represent a semantic and not a real difference, but the first question is fundamental. Either the antigen induces stable cell differentiation or it does not.