

MEMORANDUM

BRISTOL LABORATORIES

UNIT OF BRISTOL-MYERS COMPANY

FROM J. Lein

DATE November 17, 1958

TO W. Bradner

SUBJECT Dr. Lederberg's Visit

~~CC:~~

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cc:

J. Lederberg ✓

Discussions with Dr. Lederberg ranged over a wide variety of subjects with emphasis on the mechanism of antibody formation and types of metabolic analogs that might be expected to have a differential toxic effect on the cancer cell. Special consideration was given to screening methods that were consequences of the proposed mechanisms. Rather than attempting to summarize the overall discussion, I will summarize the specific technical approaches discussed which appear significant to our operations and the rationale for them.

1. Use of tritiated thymidine as a method of selecting non-dividing cells in a population of dividing ones.

This technique has been used successfully by Davis and Corini for the selection of biochemical mutants by growing the cells in a minimal medium containing tritiated thymidine. The growing cells take up the thymidine and on incubation in the cold are killed by it, leaving cells which did not take up the thymidine. The technique is analogous to the penicillin technique but appears to be a cleaner system.

2. Utilization of the "repression" phenomena to select for mutants producing end metabolites (amino acids, vitamins, etc.) in large quantities.

The proposed technique is based on the repression phenomenon observed in Davis' laboratory where the presence of specific metabolites decreases their own synthesis. This negative feed-back is, of course, useful for the over-all economy of cell synthesis. Mutants lacking this ability would be expected to produce and spill into the medium large quantities of a metabolite. The actual technique would be to grow a culture alternately in synthetic and complete medium. Mutants

lacking the "repression" phenomenon would grow more rapidly and become the dominant type in the population since the growth period in complete medium would not lead to a depletion of the synthetic enzymes. Subsequent growth in minimal medium would give these cultures a growth advantage.

3. Screening for agents capable of affecting the antibody response either at the production level or the target level.

Dr. Lederberg has adopted and altered Burnet's model of antibody formation. The modified model differs from the usual concepts in as much as the central feature is the origin by spontaneous mutation of a large variety of antibody forming cells each capable of reacting with an antigen. These cells go through an immature period where they are hypersensitive to antigens. In this state exposure to antigen suppresses antibody formation. On maturation of the immature cells, they give rise to reactive cells capable of recognizing the antigen and proliferating in response to it. This gives rise to clones of cells distinguishable on the basis that each clone is capable of producing a particular antibody. In looking for agents capable of affecting antibody formation, the agent should be given with the initial injection of the antigen as well as with the anaphylaxis-producing dose. Important agents may have the property of influencing the process whereby the antigen causes the proliferation of the mature antibody-forming cells. Alternatively, the agent may make the reactive cell take on the hypersensitive characteristics of the immature cell and induce a state of tolerance to the given antigen. Since we are screening for agents affecting this system at the present time by inducing an anaphylactic response with a second injection of serum, only a minor modification would be necessary in the procedure. Along with the sensitizing dose of antigen, should be given the material to be screened. An alternative technique would be the use of a tumor transplanted into an animal which regularly rejects the tumor because of immunological incompatibility.

Another aspect of this type of work would be to use the antigen-antibody complex itself as an inducer of skin lesions and test for agents capable of modifying the lesion. This would be aimed at finding an agent capable of affecting response of the target. Symptomatic relief of this type of reaction would be of major therapeutic importance. Such a screening technique would probably be simple enough so that large numbers of materials could be screened easily.

Dr. Lederberg also pointed out an article in the U.S.S.R. literature by Kalinin (*J. Microb. epid. & Immun.*, 86 4:515 1957) which described an agent capable of stimulating antibody formation.

4. Covering up the phosphoric acid ionizing group in metabolites and analogs to permit penetration into cells.

A great many biochemically important materials are phosphate esters. Studies with microorganisms indicate that such materials penetrate poorly if at all into cells. This has prevented trial of a very important

class of metabolic analogs. Conceivably the phosphorylated intermediates might be most interesting if they could penetrate cells. One of the simplest methods of insuring penetration would be to esterify the ionizing group. If this can be done readily, new classes of nucleotides can be tested for differential inhibition of cancer cells. It would also be of interest to determine what the effects of such a derivative of adenosine triphosphate would be since the compound itself appears to be so universally important in the energetics of biochemical reactions. A simple method of determining if the esterification is having the desired effect would be to determine if the compound was utilisable as a carbon source. Phosphorylated intermediates ordinarily are not.

5. Substitutional chemotherapy (production of chemical changes in complex mixtures of metabolites, determination if an inhibiting material is produced, isolation of inhibiting compound).

Since Dr. Lederberg will be moving from a public to a private university in January, he will be able to reconsider a more direct participation in the program. Dr. Hooper and I will look into the possibility of writing a suitable contract with the Stanford Research Institute to carry out some of this type of work. Dr. Lederberg, because of his particular interest in this field, would be a consultant for it.

Particular attention should be paid to deoxyribonucleic acid as a substrate for these reactions. Modifications of this material may be especially useful as antitumor agents.

Original signed
J. LEIN

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