GENERAL LEUCOCYTIC RESPONSE OF THE GUINEA PIG DURING THE REACTION OF ARTIFICIAL IMMUNITY IN EXPERIMENTAL TUBERCULOUS INFECTION.

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Since the early studies on immunity reactions it has been known that a definite response of the circulating leucocytes is associated with the process of active immunity. The studies of Zinsser and Tsen,\(^1\) which elaborate Bordet's original observation, furnish experimental evidence of the fact. In clinical medicine, also, it is known that a general leucocytic response accompanies the reaction of the body to bacterial infection. This response is characterized by quantitative changes in the actual number of circulating leucocytes, or in the relative proportion of the various types of cells, or in both.

Despite the fact that these observations are of long standing and are generally accepted, little is known of the actual significance of such changes. It may be stated, however, that for many infections the nature of this phenomenon is taken to indicate the degree of resistance possessed by a given individual. On the other hand, there are some bacterial infections about which there is controversy as to whether there is a characteristic leucocytic response. Hence, there is doubt whether observations of this phase of the blood picture are of value in the experimental or clinical study of such infections. Among these, active tuberculous infections are prominent. The studies of Murphy and his coworkers\(^2\) in this laboratory, and those of Webb, Williams, and Basinger,\(^3\) present evidence to show that the


resistance of animals to experimental tuberculous infection is enhanced when artificial lymphocytosis is induced before and after the inoculation of virulent tubercle bacilli. These studies support the observation of several clinicians who have noted in human tuberculous disease that the circulating lymphocytes vary directly with the degree of resistance to the disease manifested by a given individual.

In previous communications from this laboratory, evidence is presented to show that lymphocytosis may be dependent upon the activity of germ centers in lymphoid tissue, and in this sense lymphoid activity may be used as a corollary to lymphocytosis. On account of the interest and importance of these experimental and clinical observations and because the idea suggested by them is not generally accepted, it has seemed worth while to submit the subject to further experiment. We have accordingly made observations on the circulating leucocytes of guinea pigs in which resistance to virulent tubercle bacilli was raised by a previous inoculation of relatively non-virulent tubercle bacilli. We assume that animals so treated are possessed of as great a potential resistance to progressive tuberculous infection as is possible to obtain at present.

Method.

The method of protecting the animals against virulent tuberculous infection is that first carried out by Trudeau and subsequently elaborated by Baldwin, Krause, and others. A preliminary inoculation of non-virulent tubercle bacilli, Saranac Strain R1, is followed, after a proper interval of time, by an inoculation of virulent tubercle bacilli, Saranac Strain H37. As controls some animals were inoculated with Strain R1 or H37 alone. Guinea pigs were used throughout the observations.


6 Nakahara, W., and Murphy, Jas. B., Anat. Rec., 1921 (in press).

Acknowledgment is made of our appreciation to the workers at the laboratories of the Trudeau Foundation and Sanatorium for furnishing the cultures of Strains R1 and H37.
Absolute and differential leucocyte counts were made twice weekly on a number of normal guinea pigs over a period of a month. These blood specimens, as well as all others to be referred to, were obtained from a vessel of the ear. For the absolute counts the blood was diluted as usual with 3 per cent acetic acid in a diluting pipette and then shaken. 1 drop of the diluted blood was placed on the disk of a Türk hemocytometer, covered, and allowed to stand long enough for the cells to settle. Eight squares were always counted, and when the deviation of any given square was ten greater or less than the least number of cells obtained for any square, another drop was counted. The diluting pipettes were roughly calibrated before beginning the observations by making comparative counts. The same pipette was used for a given animal for each successive observation. For the differential counts blood films were prepared on cover-slips, then stained with Wright’s stain. 300 cells were counted and the percentage of each type determined from this number was applied to the absolute count to estimate the absolute differential count.

During the period of preliminary observation any animal showing an erratic tendency in the leucocyte count was discarded. The general living conditions were the same for all animals. Therefore, when the experiment was begun, conditions were standardized as far as controllable variables permit, and were maintained throughout the observations.

A number of animals were inoculated subcutaneously into the left groin with 0.2 cc. of a suspension of Strain R1 tubercle bacilli prepared so that a stained film preparation showed not more than ten individual bacilli to a microscopic field. Blood counts were continued in the manner previously described. After about 3 weeks these animals were inoculated with 0.1 cc. of a suspension of the virulent strain, No. H37, and blood counts were continued. This suspension was standardized in the same way as the R1 suspension, except that the number of bacilli was not more than two to a microscopic field.
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OBSERVATIONS.

For normal guinea pigs weighing between 200 and 250 gm. the average absolute count was 5,247 leucocytes per cc. of blood. Differential counts showed 52.1 per cent, or 2,734 of these cells, to be lymphocytes and 43.1 per cent, or 2,261, to be amphophilis (polymorphonuclear cells).

Following the inoculation of Strain R1 the reaction was characterized by a gradual but definite increase in the total leucocyte

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**Text-Fig. 1.** Composite absolute and differential white cell counts of all test animals plotted at intervals of 1 week following inoculation and reinoculation.

**Text-Fig. 2.** The percentage variation of white cells following inoculation and reinoculation. The standard average of 2,734 lymphocytes and 2,261 polymorphonuclears is indicated as zero.
counts (Text-figs. 1 and 2). At the end of 3 weeks, just before the inoculation of Strain H37, this increase was 80.5 per cent, representing an increase in lymphocytes to 60.5 per cent, while the polymorphonuclear cells, though increased absolutely, constituted 34.6 per cent of the white cells, a relative decrease of 8.5 per cent.

The reaction of the protected guinea pigs to the virulent strain, No. H37, was characterized first by a slight fall in the lymphocytes and an

Text-Figs. 3 and 4. Absolute and differential white cell counts of two individuals used in the experiment, plotted at weekly intervals before and after inoculation and reinoculation.
increase in the polymorphonuclear cells. This was observed throughout the 1st week, during which time the animals were obviously quite sick. Then there followed an exaggerated increase in the total leucocyte count, which reached a peak during the 3rd week, at which time the total percentage increase of white cells amounted to 198.5. During this period the lymphocytes increased to a point 306 per cent above their number at the beginning of the experiment, while the polymorphonuclear cells were 9.2 per cent below their normal relative

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**Text-fig. 5.** Composite absolute and differential white cell counts of individuals plotted at weekly intervals following an inoculation of a heavy suspension of virulent tubercle bacilli.

**Text-fig. 6.** The percentage variation of white cells in the counts plotted in Text-fig. 5.
average, though numerically they showed some increase. After the peak of the curve was reached, there was a decided drop in the absolute number of white cells, particularly affecting the lymphoid cells. Text-figs. 3 and 4 show typical curves of individual animals used in the experiment. In Text-fig. 4 it will be noted that the normal count shows the polymorphonuclear cells to be greater in number than the lymphocytes, an exception occasionally observed.

Blood counts on the control guinea pigs inoculated with virulent tubercle bacilli alone showed a definite increase in the circulating leucocytes. The relation between the increase of polymorphonuclear and lymphoid elements was erratic. Immediately following inoculation both types of cells increased in about the same numerical proportion. During the 2nd and 3rd weeks the lymphocytes showed a greater increase; after this time there was a progressive decrease in lymphocytes with a corresponding increase in polymorphonuclear cells.

Ten guinea pigs were inoculated with a much heavier suspension of Strain H37. The counts on these animals are shown in Text-figs. 5 and 6. They demonstrate a distinct difference in the reaction to more massive inoculation of virulent tubercle bacilli in contrast to the response to the smaller dose.

DISCUSSION.

In the observations recorded it is to be noted that evidence of lymphoid activity varies directly with the resistance to progressive tuberculous infection shown by the animals used. Such facts support the views previously quoted regarding this parallelism. It seems apparent, however, that one is not justified in attempting an explanation of this parallelism since little information is available concerning the function of lymphocytes.

The general idea of some such relation is not new, since as early as 1883 Arloing developed the notion during an attempt to bring experimental evidence to bear on the controversy of that time between clinicians and pathologists concerning the tuberculous nature of scrofula. This investigator and others who followed his lead believed that lymphoid cells possessed some specific property which was

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8 Arloing, S., Leçons sur la tuberculose, Paris, 1892.
active in the defensive mechanism against tubercle bacilli. These opinions were based largely upon observations made on the differences in the reaction of rabbits following the inoculation of material from tuberculous lymphadenitis as compared to that from other tuberculous tissues. Most of this work was done before it was known that there are bovine and human strains of tubercle bacilli, which probably accounted for the observations recorded. Renewed interest came later through the work of Bartel and coworkers who claimed that, while working on experimental tuberculous infection in guinea pigs induced by feeding tubercle bacilli, they observed in lymph nodes in which tubercle bacilli had lodged, without further evidence of invasion, a reaction characterized by a striking lymphoid hyperplasia. Because of the absence of both the characteristic mononuclear phagocyte and the giant cell, they concluded that lymphocytes alone were capable of destroying tubercle bacilli. Further experiments failed to confirm their views. It has been thought that a specific substance might be isolated from lymphoid tissue and used for artificial immunization, but the failure of experiment to support any of these ideas has led to hesitancy in the acceptance of any lead in the study of resistance to tuberculous infection which concerns lymphoid activity. However, we regard the fundamental idea in the studies initiated in this laboratory concerning lymphoid activity in tuberculous infection as totally different from any of those just mentioned. The facts brought out in these observations seem significant, and we believe that they emphasize a probable relation between lymphoid activity and resistance to experimental tuberculous infection which deserves consideration in the study of the factors of resistance to active tuberculous infection in man.

It may be said that several investigators have recorded data which show that the nature of the leucocytic reaction of individuals ill with tuberculous infection in its various forms is analogous to that recorded in this paper. For example, in generalized miliary tuberculosis and in tuberculous meningitis, in which resistance is lowest, since recovery is always improbable, there is a notable deficiency in lymphoid activity as determined by estimations of circulating lymphocytes. In progressive pulmonary tuberculosis this is also the case; on the other hand, in individuals showing an ability to control the disease, there is an increase in the circulating lymphocytes. Although there is no general agreement among various workers that these statements are correct, the disagreement is no more extensive than that concerning the leucocytic reaction in many other infections.

In this connection it seems of interest to direct attention to certain probable reasons for the differences in opinion; namely, confusion arising from observations made during different phases of a given illness associated with a particular infection, e.g. the period of typical manifestations of the infection, that of complications, and that of convalescence; conclusions drawn from data which include too few observations on different individuals; or lack of uniformity in the method of interpretation. These points are illustrated in the statement found in most text-books concerning the leucocytic reaction in general miliary tuberculosis, typhoid fever, and influenza. It is usually asserted that the leucocytic picture in the three infections is similar. However, careful analysis of reports on studies of these infections leads to quite a different conclusion. A summary of the tables in Warthin's paper11 on general miliary tuberculosis and Thayer's paper12 on studies on typhoid fever is as follows:

**General Miliary Tuberculosis.**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute count</td>
<td>4,128</td>
<td>2,872</td>
</tr>
<tr>
<td>Polymorphonuclears</td>
<td>3,777</td>
<td>1,123</td>
</tr>
<tr>
<td>(91.5 per cent)</td>
<td>(22.39 %)</td>
<td>(22.39 %)</td>
</tr>
<tr>
<td>Mononuclears</td>
<td>342</td>
<td>1,408</td>
</tr>
<tr>
<td>(8.3 per cent)</td>
<td>(30.45 %)</td>
<td>(30.45 %)</td>
</tr>
</tbody>
</table>

**Typhoid Fever.**

<table>
<thead>
<tr>
<th>Description</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute count</td>
<td>5,386</td>
<td>1,614</td>
</tr>
<tr>
<td>Polymorphonuclears</td>
<td>3,323</td>
<td>1,577</td>
</tr>
<tr>
<td>(61.7 per cent)</td>
<td>(32.18 %)</td>
<td>(32.18 %)</td>
</tr>
<tr>
<td>Mononuclears</td>
<td>2,028</td>
<td>278</td>
</tr>
<tr>
<td>(37.6 per cent)</td>
<td>(15.89 %)</td>
<td>(15.89 %)</td>
</tr>
</tbody>
</table>

The percentage estimations noted are made on the basis of a standard of 7,000 leucocytes per c. mm. of blood, with the percentage relation of polymorphonuclear cells and lymphocytes 70 and 25 respectively.

Further, the recent widespread epidemic of influenza has furnished an opportunity to gain much new information concerning the leucocyte picture found in that infection. Practically all observers affirm a leucopenia as characteristic, but there is difference of opinion regarding the relative proportion of the various cells. Some observers have noted the mononuclear elements to be especially affected, while others pay particular attention to polymorphonuclear elements. The reports which point to the mononuclear elements are based on observations made during the first 5 days of illness, while those which point to the polymorphonuclear cells are concerned with a later period of the illness. In view of the now generally recognized fact that true influenza is of only short duration (1 to 5 days) and that one deals principally with complications in an illness which continues beyond that period, it is suggested that the observations made during the early period should be of greater significance. The report of Olitsky and Gates on experimental influenza lends support to this view. While it is true that all three infections manifest a leucopenia, there are striking differences demonstrated in the degree and character of leucocyte changes.

As further emphasis to the suggestion that more consideration should be given to the possible value of lymphocytes in the defensive mechanism against tubercle bacilli, attention is called to the constant presence of these cells in the local reaction product resulting from tissue injury by tubercle bacilli. Among the numerous investigators who have studied the histogenesis of the tubercle, there has been a tendency by the followers of Baumgarten to disregard the lymphocytes entirely, while those following Metschnikoff have regarded them as progenitors of the characteristic mononuclear phagocytes found in the lesions. It must be stated, however, that all these workers have concerned themselves principally with the controversy as to whether fixed or wandering cells enter chiefly into the reaction.

More recently, however, Wallgren in a study on experimental tuberculosis of the liver in rabbits, emphasized the part played by

lymphocytes in the reaction of the tissues of this organ to tubercle bacilli, while von Fieandt, in a similar study on the meninges, found little evidence of participation by lymphocytes in the process. These observations are of interest in connection with the established fact that different tissues of a host vary in their resistance to parasitic invasion. Soper has demonstrated in the liver of the rabbit a relatively high degree of natural resistance to tubercle bacilli, and it is well known that the meninges are very susceptible to these parasites. Whether or not the success of such a local resistance may be related to a combined activity of the two types of mononuclear cells found in the product of reaction can only be conjectured at this time. Further elaboration of this point must be delayed until methods of experiment are available which will permit its investigation. In the meantime it would seem more plausible to assume that the presence of lymphocytes in these reactions is a purposeful phenomenon, as is believed for other types of cells in various reaction products following tissue injuries. Particularly is this true in the light of available information regarding the factors concerned in the mechanism of immunity in tuberculous infection. There is almost unity of opinion that as far as cellular and humoral elements are concerned, the former are conspicuous while the latter are relatively inconspicuous.

SUMMARY.

Guinea pigs have been rendered relatively immune against infection with virulent tubercle bacilli by preliminary inoculation with a suitable quantity of avirulent tubercle bacilli. Blood counts on these animals show that associated with the immune reaction there is a definite general leukocytic response characterized by an absolute increase in the total count, with an absolute and relative increase in the lymphocytes. The period of greatest activity coincides with that known to be the period of greatest reaction, based on anatomical evidence during the course of infection following this method of immunization. Moreover, blood counts made on animals inoculated with avirulent tubercle bacilli alone show an increase in the circulating

lymphocytes during the period of greatest reaction to the infection, while blood counts on guinea pigs inoculated with virulent bacilli alone show an erratic course in which the polymorphonuclear forms are much increased, though not regularly so.

These results indicate a parallelism between lymphoid activity and resistance of the animals to tuberculous infection, and suggest an association of lymphocytes with the factors determining this resistance, a relation which warrants consideration of the blood picture in the clinical study of tuberculous infection.