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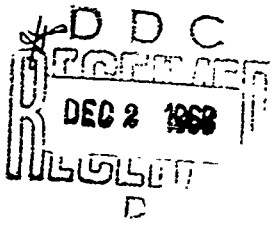

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Antibody in the leg lymph following active immunization.

by H. Onaka

Arbeiten aus der dritten Abteilung des Anatomischen Institute der Kaiserlichen Universitat Kyoto, Series D, No. 7: 151-171 (1938).

In the wake of Shinzaburo Takahashi's report (this series, No. 4: 90, 1934; 5: 1 and 9, 1935-36), I took up the question of antibody in the leg lymph. Murakami (Nippon-Biseibutsugaku-Byorigaku-Zasshi, 30: 211, 221, 1937), under the supervision of Prof. Kimura, found antivibro agglutinin in the leg lymph A and E in connection with the active immmunization of the rabbit with Vibrio cholerae. By the modification of the method for obtaining lymph, we were able to almost completely prevent the admixture of blood during withdrawal of lymph. Thus the lymph of the vas afferens et efferens Lgl. popliteae contains almost no erythrocytes. Horii and Oya consider hemal admixture as probable in case of the presence of erythrocytes in excess of 1%. Polymuclear leukocytes, if present in the lymph obtained, always indicate hemal admixture in normal animals.

I was able to circumvent hemal admixture to the lymph used in my antibody research by careful examination of slide preparations. For the time being I should like to ignore the ticklish question whether all of the lymphatic fluid and the substances dissolved therein directly emanate from the blood. We know from the experience of our colleagues that certain soluble salts are demonstrable in the leg lymph shortly after instillation in the blood stream. Murakami's tests make it probable that antibody injected into the blood stream is capable of moving from the blood into the lymph.

1) Agglutinin against Bacterium typhosum.

Large, well-nourished rabbits are intravenously inoculated, once a week, with doses of 0.5, 1.0 and 1.5 ccm of a vaccine, of which 1 ccm contains one standard loopful of a fresh agar culture of Bacterium typhosum. One week after the last injection of vaccine, the lymph is withdrawn from the vas afferens et efferens Lgl. popliteae.

I have determined previously that an agglutination reaction of the lymph E and of blood serum in normal animals occurs up to a maximum of 40-fold dilution.

2) Agglutinin against Bacterium pullorum.

I have immunized the second series of test animals (No. 9-20) with a vaccine of Bacterium pullorum (Taiwan, No. 37) in the analogous manner as the aforesaid rabbits.

The lymph was withdrawn 1-2 weeks after the last injection.

As is evident from the table, an enormously high agglutination titer is established in the lymph as well as the blood serum, and this in a descending sequence in the blood, the out-flowing E lymph and the in-flowing lymph A.

In the case of the third group of rabbits (No. 21-40), I tested the agglutination values at different times (table III).

According to the latter, the agglutination of bacilli in the lymph as well as in the blood is not particularly distinct up to the 4th day following a single injection of vaccine; it only rises to a rather considerable degree after one week. After the third injection it has also increased gradually in the lymph. Owing to this behavior, I had at first tended toward the opinion that the agglutinins contained in the lymph are produced at least in the region of the lymphatic source, if not in the lymphatic gland itself.

During the course of time the agglutinins gradually disappear from the lymph. The abatement of the agglutinative effect becomes conspicuously evident in the fifth week after the last injection of vaccine; after 128-171 days it is barely demonstrable in the lymph, as little as in the blood.

Above tests show that the quantity of agglutinin in the blood serum is always greater than in the leg lymph. This fact permits the assumption that the agglutinin in the leg lymph is capable of penetrating, by filtration, from the circulating blood into the tissue fluid. Murakami claimed to have proved this in connection with leg lymph after intravenous injection of antivibrio serum. We must be careful, however, not to draw the same conclusion prematurely, since no proof has yet been presented in support of the assumption that the tissue from which the leg lymph emanates, is incapable of antibody production. Reutman has pointed out long ago that the venous blood of the spleen contains more antityphous antibody than the circulating blood, leading him to conclude that the spleen must be considered as their source. It is conceivable that the lymphatic gland also is involved in the production of antibody, especially a large gland such as the knee lymph gland of the rabbit. In order to examine this question more thoroughly, I injected the vaccine into the skin of the toes instead of into the auricular vein and obtained the results tabulated in table IV.

Initially, after subcutaneous injection of vaccine, the agglutinative effect in the out-flowing lymph E is greater than in the blood. Murakami has obtained identical results in similar tests with typhus bacilli, and has reached the conclusion that the regional lymph gland produces the specific agglutinin after injection of vaccine into the source. Unfortunately he did not examine the lymph of the other leg. I have examined the lymph of both legs simultaneously and have found quite a large amount of agglutinin in the lymph which flows out of the knee lymph gland of the other leg (into the digital skin of which no vaccine had been injected). This surprising result leads me to believe that the production of agglutinin in the tissue surrounding the source, which takes place after injection of vaccine into the digital skin, is a partial manifestation of a general reaction of the lymphatic tissue. Antigen instilled in the distal end of the leg is rapidly

absorbed via the lymphatic channels, reaches the blood circulation (as confirmed by many colleagues by means of certain soluble and insoluble substances), causing a general reaction of the formative tissue. For this reason the formation of antibody has also risen enormously in the knee lymph gland of the leg that did not receive vaccine. Moreover, it may be assumed that the substance injected into the digital skin very rapidly passes through the knee gland, and that a sustained rejection of the injected material solely in the regional gland is improbable. The antibody produced in the lymphatic gland is passed into the blood circulation via lymphatic channels and are stored there for accumulation. Thus the agglutinative influence in the blood may gradually exceed that of the lymph.

Observations concerning group agglutination of the leg lymph of rabbits immunized with pullorum against typhus bacilli are listed in table V. In this connection a group reaction (even though not very strong) is established in the leg lymph as well as in the blood, the reaction against typhus bacilli being very strong in the blood serum, moderately strong in the lymph E emanating from the knee gland and weak in the in-flowing A. The behavior is therefore identical with pullorum.

Agglutinin was also demonstrated in the leg lymph after peroral immmunization. The results are tabulated in table VI.

Salomonsen and Madsen (Compt. rend. de l'Academie des Sciences, 126:1229, 1898) report on the influence on antibody production of toxins which affect the autonomous nerves; thus, in the case of a horse which had been treated with diphtheria toxin for some time, the antitoxin content of the serum increased distinctly following injection of 1.4 g pilocarpine; no decrease in the antitoxin content of the serum was noted in the same horse after instillation of atropine. Rosenthal and Holzer (Berl. klin. Wochenschr. 1921, 675) later reached the conclusion that pilocarpine is able to inhibit the agglutinin production in rabbits immunized with typhus bacilli. Litarczek (Zeitschr. f. ges. exp. Med. 46: 656, 1925) and Butjagin (cited in Bielig and Kolle's manual), on the other hand, claim an increase in agglutinin production with pilocarpine. Joachimoglu and Wada (Arch. f. exp. Pathol. u. Pharmacol. 93:269, 1922) were unable to demonstrate a notable effect of pilocarpine on the quantity of serous agglutinin against typhus bacilli in rabbits, although the quantity seemed to decrease somewhat with atropine.

I have now observed the effect of atropine and pilocarpine on the quantity of agglutinin in the leg lymph.

Following injection of pilocarpine, the agglutinin content (concentration) is lowered in the leg lymph as well as in the blood serum. As yet I have not been able to determine the cause of the decrease in agglutinin after instillation of pilocarpine. Gamus and Gley (Arch. de Pharmacodyn., I: 487, 1895) showed in connection with dogs that pilocarpine decreases the secretions of lymph from the ductus thoracicus in considerable amounts, but that the flow is almost instantaneously increased by an injection of atropine. It is still not clear, however, whether the decrease in the flow of lymph is connected with the lowering of the agglutinin concentration.

Atropine does not seem to effect the agglutinative effect of the lymph.

Table I.

Agglutination, tests with typhus bacilli.

The animals received 0.5 ccm of a vaccine, containing one standard loopful of B. typhosum per 1.0 ccm, in the first week, 1.0 ccm in the second, and 1.5 ccm in the third week. The leg lymph and the blood were withdrawn one week after the last injection.

Kaninchen Nr. — rabbit #.
Verdünnung der Lymphe oder des Blutserums — dilution of lymph or blood serum.
Röhrchennummer — tube #.

Table II.

Agglutination, tests with B. pullorum.

(Same as table I, but different pathogen)

Table III.

Agglutination, tests with B. pullorum.

Vaccinal doses and withdrawal times vary.

Table IV.

Agglutination, tests with B. pullorum.

Vaccine was injected into the digital skin of one leg. dext.: right knee lymph gland. sin.: left knee lymph gland.

Table V.

Group agglutination; the animals are immunized with three injections of B. pullorum vaccine.

Table VI.

Agglutinin after peroral immunization with B. pullorum.

Table VII.

Influence of pilocarpine on the agglutinin content of the lymph and blood.

Table VIII.

Effect of atropine on the agglutinin content of the lymph and blood.