Experimental Lupus Erythematosus in Animals

By

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It has been known for many years that immune reaction may occur against the foreign proteins, when entering the body. In the era of sero-therapy these reactions were well known. These immune reactions were caused by intrance of one species's protein to another's body.

Landsteiner and Wiener (1) in 1940 with their extensive work on blood groups produced immunity in the same species by injecting red blood cell of Rh..monkeys in one another. Today erythroblastosis is well known in human. Another step in this direction was put ahead by Kabat and al (2) and Whitebsky and al (3) in produsing immunity to "self" tissue by developing "allergic myeloencephalitis" in rabbit and Hashimoto-like disease in dogs and rabbits.

On the other hand, interreaction of lupus erythematosus plasma with nucleoproteins and especially DNA, was manifested by Hollman, Kunkel, (4) Friou and Associates (5, 6) using the fluorescent antibody showing that the factor in lupus serum which reacts with nuclei also reacts with desoxyribo-nucleoproteins (nucleohistone), from calf or rabbit thymus and with artificial complexes of D.N.A. with histone. And the same investigators showed the affinity of globulin component of lupus patient for nuclei with the same technic. In the light of these experiments the possibility of immune state to nucleoprotein as the cause of L.E. is evoked. In the following report attempt has been made to produce this immunity in laboratory animals (Guinea-pigs).

* This work was done when a resident at Creighton University.
Antigens

Chromosin: Chromosin was extracted from guinea pigs liver by the procedure of Mirsky and Pollister (7).

Method: All operations were carried out in a cold room. Remaining at 32 to 35°F. 500 Gm of liver tissue were removed from guinea pigs and within one hour after removal were cut into small pieces and suspended in one liter of normal saline (0.14 M. NaCl). To cut down the foam of the suspension a few drops of octyl alcohol were added and the liver suspension was minced in a high speed electric mixer for one minute. The suspension was centrifuged for 10 minutes at 8000 R.P.M. The turbid supernatant was discarded and the residue was again suspended in the same solution and was again minced with an electric mixer. After centrifuging at 8000 RPM the supernatant was discarded and the residue was washed three more times. By this time the supernatant became much less turbid. The residue was suspended in 0.14 M. NaCl to make a total volume of 750 cc. 750cc of 2M NaCl was added to this solution. This suspension was mixed in a high speed electric mixer for three minutes, and the mixture was stirred rapidly for 16 hours. The viscous solution was centrifuged for 60 minutes at 8000 RPM. The supernatant was obtained and by pouring into 6 volumes of distilled water the chromosin precipitated. To the precipitate, an equal volume of 2M NaCl was added and by adding 1 M. NaCl to it. The total volume was brought to 450cc. The solution was vigorously stirred until the chromosin dissolved, and by centrifuging at 8000 RPM the supernatant was added to 6 volumes distilled water and the precipitate, a fibrous material removed and dissolved in 1 M. NaCl, and kept at 32°C for use as chromosin. No attempt was made to purify the DNA.

Freund Adjuvant: (Incomplete) a nine part mineral oil and one part Arlcel was emulsified for half an hour. A whitish emulsion was obtained.

Antibody Absorbed Antigen:

20cc. of citrated blood was removed from antecubital vein of a female patient suffering from lupus erythematosus. Plasma was removed, and 2cc. of chromosin was added to it and incubated at 37°C for 30 minutes.

According to Holman (5), by this procedure, 50 percent of DNA would be removed by patients globulin (presumed antibody in lupus erythematosus). This solution was kept at freezing temperature. The same procedure was done to the plasma of group I guinea pigs (below).

Recipient:

24 guinea pigs (12 female) 300 to 400 Gm each were divided into five groups and subjected to the following procedures.

Group I - six guinea pigs received 4 injections of chromosin in one M. NaCl weekly. (i.g., subcutaneously, thigh). Two of these animals were sacrificed at the end of one month and the remaining 4 kept for another month.

Skin Reactions:

48 hours after second injection the site of injection became red and indurated. In one of the animals transient swelling and tenderness of joints became apparent. After seven days, in three pigs, the skin at the site of injections was thick and indurated. Animals were sacrificed 6 to 8 weeks after the first injections and LE preparations were done by “Rotary Method” (2). Also blood smears were obtained. Hb determinations were done. Hb level was 90 to 120% of normal value in all animals. Blood smears did not show any deviations from normal values and LE preparations were all negative.

Group 2 - six guinea pigs received 4 injections of chromosin emulsified in “Incomplete” Freund adjuvant in weekly intervals. Two remaining 4 kept for another month.

Response:

Swelling, tenderness and rubor of joints appeared in all guinea pigs after second to third week (10 to 21 days). This reaction was transient and lasted less than 72 hours at the most.

Induration of skin with slight edematous wheels appeared in two determinations. Examination of blood smears did not reveal any change. LE preparations were negative.
Sections of liver in two animals did not show any change.

Group 3. Plasma of lupus patients was incubated with chromosin for 30 minutes at 37°C and mixed with Freund adjuvant. The mixture was emulsified. Four guinea pigs received four injections of weekly interval.

After the second injection, the skin became inflamed and in two animals necrosis of skin occurred. After third and fourth injection skin laceration occurred in all animals. The laceration stayed between four and fifteen days. Hemoglobin and blood smears remained normal. LE preparations were negative in all animals.

Group 4. Four guinea pigs of this group received injections of half cc Freund adjuvant of weekly intervals.

The location of injections became indurated and thick. After second injection, (10 to 21 days after the first injection) transient arthritis occurred, in the form of swelling, tenderness and redness, in all animals. Hemoglobin remained normal. LE preparations were negative.

Group 5. Plasma of group 1 and 2 were incubated with chromosin for 30 minutes at 37°C. Then the solution was emulsified in Freund adjuvant. Four injections of 0.4 cc, was given to four guinea pigs of this group. Skin laceration occurred in all animals after second or third injection. Hemoglobin remained normal. LE prepartations were negative.

DISCUSSION:

Biett was the first who mentioned the disease as “Erythema centrifuge” in 1828. At this time, the main attention was toward dermatologic manifestation of the disease. In the following four decades, Habra, Cazenali and Kaposi with their individual approaches to the described disc-like patches, differentiated it from lupus vulgaris and in 1872, Kaposi mentioned systemic manifestations of the disease (erysipelas perstans faciei). Sir William Osler in 1895 described the course of the disease and its fatal outcome. He also described visceral involvement of the disease. His description of the disease was the first step toward the departure of the disease from dermatological books, as he pointed out the development of the disease without cutaneous lesions.

Jadassohn in 1904 reviewed the disease and referred to the frequency of arthritic, eruptions and renal involvements. In the 1920, the interest in the disease was renewed. Keefer and Felty added leucopenia and thrombocytopenia to the already described symptoms. Endocardial and valvular involvements in at least two cases were described by Libmann and Sacks in 1924 and later, Gross comprehensively detailed those changes.

Buehr, Klemperer and Schifrin reviewed 23 cases thoroughly and described the vascular lesions in 1935. They described the microscopic changes of pathologic tissue and referred to “a peculiar hyaline thickening of glomerular tufts”.

In 1942 Klemperer, Pollack and Buehr reached the conclusion that the fundamental changes in SLE were manifested primarily in the collagenous tissue of the body.

Photosensitivity has been recognized for some time as a Precipitating factor in the production of local and systemic manifestation (28 & 32) and “avoidance of exposure to sunlight was recommended” (29).

A search of the blood and urine for porphyrin has given negative results (30).

Incidence of false positive serology for syphils has been pointed out by many investigators (28, 10, 13). Rein reported 32% positive reaction in 1178 cases. The incidence increases with the acuteness of the disease (29).

Serum protein changes were noticed and described by Coburn et al (33) and others (5). These changes are non-specific and are manifested by an increase in gammaglobulin. More specific is the increase of hexosamines to two to three folds of normal values (16).

Geographical distribution of lupus was studied by Gahan (19), in 1942 in several cities of the United States (Boston, New York, St. Louis and others) and eight different countries of Europe, North and South America. There was no significant differences. He pointed out, “if O.4% is taken as the incidence of lupus erythematosus in the United States, there is no appreciable difference between the relative frequency of the disease”. 

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Hypothesis of Lupus Erythematosus

The hypothesis that lupus erythematosus is caused by the presence of antinuclear antibodies (ANA) has been widely accepted. These antibodies are produced in response to self-antigens, leading to immune complex formation and subsequent tissue damage. The precise mechanism by which ANA leads to disease remains unclear, but it is thought to involve activation of the complement system and recruitment of inflammatory cells to the affected tissue.

Conclusions

The experimental model of lupus erythematosus provides insights into the pathogenesis of the disease. It is characterized by the presence of ANA and the development of autoantibodies targeting various nuclear antigens. The model is useful for studying the immune response and the role of cytokines in the disease process. Further research is needed to understand the underlying molecular mechanisms and to develop effective therapeutic strategies.
Summary

(1) Deoxyribonucleic acid was obtained from the liver of guinea pigs.
(2) Five groups of guinea pigs were subjected to DNA, DNA patient's plasma DNA, patient's plasma with Freund's adjuvant, DNA, Freund's adjuvant, and guinea pigs' plasma with Freund's adjuvant. (3) Skin lesions, general manifestations, and laboratory studies have been reported (4). A review of the literature has been done.

Bibliography

15) Charles C Thomas series 42, 106.