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Experimental Lupus Erythematosus in Animals ✿

By

H. Vahabzadeh, M. D.

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 myelo-encephalitis" 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 Asscciates (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

Chromsin: 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 remainiag 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 reridue 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 shree 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° F. for use as chromosin. No attempt was made to purify the DNA.

Freund Ajuvant- (Incomplete) a nine part mineral oil and one part Arlacel 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[4], by this procedure, 50 percent of DNA would be removed by patients globulin (pressumed 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).

Recipients

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. (½cc. subcutanously-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 sight 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 sight 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" [12]. Also blood smears were obtained. Hb determinations were done. Hb level was 80 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 trancient 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 iejection 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) trancient 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 ½cc. 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 dermatoligic manifestation of the disease. In the following four decades, Habra, Cazenanl 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 faceii). 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 arthicular, erouts and renal involvements. In the 1920, the interest in the disease was renewed. Keefer and Felty [9] added leucopenia and thrombocytopenia to the already described symptoms. Endocardial and valvular involvements in at least two cases were described by Libmann and Sacks [10] in 1924 and later, Gross [11] comprehensively detailed those changes.

Baehr, 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 Baehr reached the conclusion that the fundamental changes in SLE were manifested primarily in the collagenous tissue of the body [13].

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 giveo negative results. (30).

Incidence of false positive serology for syphilis has been pointed out by many investigators [29&31]. Rein[31], reported 35% 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[35] and others[8]. These changes are non-specific and are manifested by an increase in gammaglobulin. More specific is the increarese of hexosamines to two to three folds of normal values [36].

Geographical distribution of lupus was studied by Gahan[15]. 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 wes no significant differances. He pointed out, "if 0.4% is taken as the incidence of lupus erythematosus in the United States, there is no appreciable difference between the relative frequency of the disease".

French and Viennese authors (Kren and Lowenstein) were the first who suggested tuberculosis as the cause of the disease. Even they reported positive blood cultures as high as 77% and recovered tubercle bacilli from sking lesions. Gold sodium thiosulfate was given for treatment by Shamberg and Wright to eradicate tuberculosis. This idea was valid for more than two decades when Ameaican investigators strongly opposed it. H. Keil (17) was impressed by lack of evidence of tuberculous lesions in 20 autipsies. J.F. Madden[18] reported "innoculation of animals with materials from lesions of chronic lupus erythematosus for the production of tuberculosis" with negative results.

He explained the higher incidence of tubeculosis in these patients, as was estimated at the time, by the increased suseptibility of the SLE patient to tuberculosis. R.A.O'Leary[19] also prerented evidence against tuberculous theory and suggested bacterial infection as responsible cause. He referred to the work of Welsh as presumptive evidence that SLE being caused by bacterial infection and possibly by streptococcus.

Rantz, et al[33] determined the level of ASOT and AHT in sufferers of lupus. Their values were within normal linits, High incidence of disease in females provoked attention to the endocrine system. It was noted that menses coincidence with "flare up" of the didease[32]. Although natural menopause has no effect on the course of the disease,[34] and therapeution of women has not been followed by alleviation of systemic symptoms[29]. Also endrogen therapy has not been effective.

Introduction of LE cell and the method of demonstration of LE cell by Hargraves[20] was a new and strong step toward understanding and diagnosis of the disease. Very soon the "LE - like cells" were seen in multiple myeloma[21], amyloidosis[22], hepatitis, CA. of the colon, allergic reactions to penicillin, during hydralazine therapy and several other occasions. Increase in the incidence of the disease after introduction and wide use of sulfonamides in 1933 and thereafter and mainfestation of LE phonemenon with penicillin and apresoline therapy supported the theory of hypersensitivity to chemical and organic materials. Mortinsen, et al[23] expressed the belief that systemic lupus is an allergic reaction to one of a variety of antigenic agents. Rich[14] also supported the

hypothesis of hypersensitivity. Hydralasine not only causes the LE-like cell, but also produces the clinical manifestations of the disease in the form of fever, rheumatic syndrome, leukopenia and plasma protein changes[25]. These manifestation occur ususally after long term therapy with high doses[25,25] but occasionally it occurs with a moderate amount of hydralazine[27].

Occasionally, LE cells have been found even without any clinical or other laboratory maifestations. These manifestations are transcient and although some required steroid therapy, non was durable of fatal. Baggenstoss[24] has opposed the hyperergic hypothesis and points out, "although the appearance of many of the lesions of lupus erythematosus resembles hyperergic or allergic inflammatory reactians, one is not justified on the basis of morphologic evidence alone, to consider lupus erythematosus as a manifestation of hypersensitivity".

CONCLUSION

With the work of Miescher and Holman and Kunkel[4] and Friou[5,6] and others in demonstrating the presence of antidesoxyribonucleic acid globulins in patient's plasma, attention was drawn toward the possiblity of autosensitization of the body to self-nucleoproteins. In the present study, using the nucleo-histone of guinea pigs, no manifestation of the disease could be produced. The subjects continued gaining weight. Hb levels remained within normal range and LE prepartations were negative. Sections of the liver in two animals did not show any abnormality. Joints and skin manifestations could very likely be allergic and foreign proteins. To consider the fact antidesoxyribonucleic acid has been demonstrated in the patients' plasma, but provoked sensitivity has not been successful one would reach the conclusion. That, it is not the exposure of the body to nucleo proteins that activates the defence mechanism releasing antibodies in thesera. It could be hypothesized that there is a disturbance in the regulatory mechanism in detecting "self" from "unself" probably by a group of abonrmal cells, releasing globulins with antinucleoprein property.

Summary

(1) Desoxyribonucleic acid was obtained from the liver of guinea pigs,

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

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