NODULAR VASCULITIS: IMMUNOFLUORESCENT STUDY

7S GAMMA-GLOBULIN AND COMPLEMENT (β1c-GLOBULIN) IN LESIONS OF NODULAR VASCULITIS*

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The possibility that nodular vasculitis (N.V.) (1) may be the result of an immunologic reaction has been indirectly suggested by a series of clinical (2), histological (3, 4, 5), serological (4, 6) and therapeutic findings (4, 6). However, there is at yet no conclusive evidence of the immune nature of the disease.

The immunofluorescent technic has made it possible to demonstrate the presence of gamma-globulin and complement at the tissue level in several immunological diseases (7, 8, 9, 10). Hence, it was thought that the immunofluorescent technic might provide a valuable tool for the study of nodular vasculitis.

In this work evidence is presented which indicates that in the vascular lesions of N.V. there is a simultaneous deposit of gamma-globulin and a component of the complement system (Beta 1c-globulin). These findings give further support to the hypothesis that an immunological pathogenesis is related to the disease.

MATERIALS AND METHODS

Eight patients with typical clinical and histological manifestations of N.V. were included in this study. The tissues were obtained by punch biopsy performed on a characteristic nodule in each case. Specimens of normal skin served as control material.

The tissue sample was divided into two suitable size blocks; one of them was frozen in dry ice and immediately cut at −20° C. at a thickness of about 5 microns. After formalin-fixation, the remaining block was stained with hematoxylin and eosin, and P.A.S.

Immunofluorescent studies were performed on the frozen sections either without fixation or after acetone fixation (5 minutes at room temperature). In some instances, 10% buffer-formalin (pH 7.2) fixation for 2 minutes was also used. These sections were studied by the direct immunofluorescent technic with the antisera to be described below.

As control for the immunofluorescent staining methods, the following procedures were performed in every case: microscopic observation of the section without any treatment (spontaneous fluorescence) and incubation of the sections with unlabelled antiserum followed by staining with labelled antiserum (blocking technic). In addition, staining with human anti-albumin and rat anti-gamma-globulin conjugated with fluorescein isothiocyanate was carried out in all cases as further control of specificity.

Antisera: Anti-plc-globulin: This antiserum was obtained by immunizing rabbits with pure plc-globulin prepared as described by Müller-Eberhard (11). One preparation of rabbit anti-plc-globulin was a generous gift of Dr. Charles L. Christian (Presbyterian Hospital, New York, N.Y.). This antiserum revealed a faint contaminant of 7S gamma-globulin on immunoelectrophoresis. After absorption with purified 7S gamma-globulin this antiserum gave only one arc with the characteristic shape and location of the plc-globulin when tested with normal fresh serum and the typical plc-pla arc when aged serum was used (Fig. 1).

Anti-7S-gamma-globulin: This antiserum was obtained by immunizing rabbits with pure 7S-gamma-globulin prepared as described by Müller-Eberhard (11). One preparation of rabbit anti-β1c-globulin was a generous gift of Dr. Charles L. Christian (Presbyterian Hospital, New York, N.Y.). This antiserum revealed a faint contaminant of 7S gamma-globulin on immunoelectrophoresis. After absorption with purified 7S gamma-globulin this antiserum gave only one arc with the characteristic shape and location of the β1c-globulin when tested with normal fresh serum and the typical β1e-β1a arc when aged serum was used (Fig. 1).

Anti-β1S-gamma-globulin: This antiserum was prepared by immunizing rabbits with pure 19S gamma-globulin. The protein was prepared from normal human serum by column chromatography on D.E.A.E.-cellulose as described by Vaerman et al. (12).

Anti-19S-gamma-globulin: This antiserum was prepared by injection of rabbits with 19S gamma-globulin obtained from the serum of a patient with Waldenström’s macroglobulinemia. The protein was isolated by repeated precipitation with distilled water and further purification on a column of Sephadex G-200.
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Immunoelectrophoretic analysis of the anti-beta 1c globulin serum. In both wells: fresh normal human serum. Upper trough: anti-whole human serum. Middle trough: anti-B1c-globulin. It can be seen that the anti-B1c-globulin serum reveals only this protein.

Anti-albumin: This antiserum was also prepared in rabbits by injection of purified human albumin commercially obtained from Cutter Quimica Argentina.

Anti-rat gamma-globulin: The preparation of this antiserum has been described previously (6).

Preparation of conjugated antisera: All the rabbit sera were precipitated once with 15 per cent sodium sulfate. After dialysis against buffered saline pH: 7.2, the globulins were conjugated with fluorescein isothiocyanate as described by Marshall et al. (13). The globulins were freed of excess fluorescein by filtration through Sephadex G-25. Before use, all antisera were controlled by immunoelectrophoresis, and absorbed with guinea pig liver powder and sometimes conveniently diluted in order to remove all the non-specific fluorescence.

Optical system: This has been described in detail in previous publications (14).

RESULTS

Hematoxylin and eosin and P.A.S.-stained sections revealed typical lesions of N.V. in every case (Figs. 2 and 3). The histopathological diagnosis was made following the generally accepted criteria described by Duperrat and Monfort (3).

When N.V. lesions were stained with anti-beta 1c-globulin a bright fluorescence was found mainly on the fibrinoid necrosis sites of the vessels and on damaged perivascular tissues. Staining with anti-7S-gamma-globulin paralleled the pattern observed with the anti-beta 1c-globulin conjugate (Figs. 4 and 5). It should be pointed out that when the skin samples were obtained from recently developed lesions the staining with anti-beta 1c-globulin and anti-7S-gamma-globulin revealed a very bright fluorescence.

The fluorescence was completely inhibited when the blocking technic was used. None of the cases demonstrated an obviously positive result when the tissues sections were stained with the anti-19S conjugate.

No specific fluorescence was observed after parallel sections were treated with anti-human albumin and anti-rat gamma-globulin.

Normal skin did not stain with either anti-beta 1c-globulin or with anti-7S-gamma-globulin conjugates. However, some increase of the yellowish autofluorescence of stratum corneum, elastic fibers and other normal autofluorescent structures was observed when the sections were treated with the fluorescein labelled antisera.

DISCUSSION

The pathogenesis of N.V. has not been clearly established. Several authors have suggested that the development of this disease is related to some type of immuno-allergic disorder. This interpretation is supported by the following evidence: histopathological changes of the type usually seen in well characterized allergic reactions (3, 11), hypersensitivity to certain drugs and bacteria (2, 4) and high titers of antistreptolisin (6). The favorable response to corticosteroid therapy (3) adds further support to the allergic hypothesis. However, none of the foregoing findings can be

Fig. 1. Immunoelectrophoretic analysis of the anti-beta 1c globulin serum. In both wells: fresh normal human serum. Upper trough: anti-whole human serum. Middle trough: anti-B1c-globulin. It can be seen that the anti-B1c-globulin serum reveals only this protein.

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accepted as definite evidence in favor of an immune pathogenesis.

Notwithstanding its limitations, the immunofluorescent method has made it possible to obtain more precise evidence favoring the hypothesis that immune reactions are involved in the pathogenesis of a complex group of diseases. Thus, it has been established that the demonstration of tissue bound gamma-globulin and/or serum complement components provides one of the most important evidences of immunological phenomena in the lesions of certain diseases (7). A number of investigations have revealed the usefulness of this approach to the study of systemic lupus erythematosus (7, 10), periarteritis nodosa (9), amyloidosis (7, 9), experimental serum sickness (10), nephrotropic nephritis (8) and other diseases in which an immunological response seems to be closely related to their pathogenesis (9).

Using this approach we have demonstrated the simultaneous occurrence of 7S-gamma-globulin and beta-1c-globulin in the vascular and perivascular lesions that characterize N.V. Since a review of the work of Lachmann et al. (7) on the in vitro conditions of fixation of beta 1c-globulin has revealed that this globulin, when observed in an untreated tissue section represents in vivo complement fixation, it can be strongly suggested that N.V. lesions are the result of, or at least closely related to, an immune mechanism.

That the deposit of 7S-gamma-globulin and beta 1c-globulin at the level of the lesions does not represent a non-specific phenomenon is shown by the results obtained in the control experiments. Thus, no positive reactions were found when normal skin was tested with the same antisera; the fluorescence could be clearly blocked by the previous treatment of the section with unlabelled antisera, and no albumin could be demonstrated by an immunofluorescent procedure similar to the one used with the other antisera. In addition, the antisera used were highly specific as revealed by the immunodiffusion studies described above.

In summary, even in view of the careful evaluation that requires the demonstration of 7S-gamma-globulin and beta-1c-globulin at the tissue level as critically discussed by Lachmann et al. (7) our observations strongly suggest a direct relationship between an abnormal immune reaction and the pathogenesis of the lesions observed in nodular vasculitis.

SUMMARY

The immunofluorescent technic was used to determine the presence of 7S-gamma-globulin and C'1beta-globulin in the skin lesions of nodular vasculitis.

By the use of specific antisera it was possible to demonstrate that 7S-gamma-globulin and beta-1c-globulin are present at the sites of fibrinoid necrosis of the small and medium vessels and on damaged perivascular tissues.

Notwithstanding the limitations of the immunofluorescent method, our findings strongly suggest a direct relationship between an abnormal immune reaction and the pathogenesis of the nodular vasculitis.

REFERENCES

11. Müller-Eberhard, H. J., Nilsson, V. and Arons-

