Delayed-Type Hypersensitivity Skin Reactions in Congenital Afibrinogenemia Lack Fibrin Deposition and Induration

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ABSTRACT Induration is a characteristic feature of delayed-type hypersensitivity skin reactions and is the usual measure of their intensity. The precise basis of induration has not been established, although activation of the clotting system with consequent fibrin deposition has been clearly implicated. In this study, two subjects with congenital afibrinogenemia, a genetic defect in fibrinogen synthesis, were skin tested with standard microbial antigens: streptokinase-streptodornase, monilia, mumps, and tuberculin purified protein derivative. One positive delayed reaction from each subject was biopsied at 40–48 h and compared with 23 biopsies of similar skin tests in normal volunteers.

The eight skin tests in the afibrinogenic subjects lacked induration, although the erythema was similar in size (10–34 mm in diameter), intensity, and time-course to those in normals. Biopsies from the two strongest reactions from the afibrinogenemic subjects showed a typical perivascular mononuclear infiltrate. No more than traces of fibrin/fibrinogen were detected by immunofluorescence, in striking contrast to the abundant fibrin/fibrinogen deposition in 23 positive, indurated reactions in normal subjects. These findings indicate that fibrinogen itself is essential for the development of induration in delayed-type skin reactions in man. As judged by 1-μm sections and fluorescence, this is probably a result of the formation of an extravascular fibrin gel.

INTRODUCTION

Cell-mediated immunological reactions are believed to play an important role in a variety of diseases in man. Delayed-type hypersensitivity skin tests, epitomized by the tuberculin reaction, have provided a useful model for analyzing the pathogenetic mechanisms of these cellular reactions in vivo (1). A central feature of classic delayed-type skin reactions is induration, yet there is some controversy concerning its pathogenesis (2–4). Specific evidence that fibrin or fibrinogen is essential for the development of induration is provided by this study of delayed-type skin reactions in two patients with congenital afibrinogenemia, a rare, isolated genetic defect in fibrinogen synthesis (5).

METHODS

Patients. C.M. is a 32-yr-old Caucasian male with afibrinogenemia who has been reported (6, 7). His plasma does not form a visible clot, although the presence of fibrinogen has been detected at minimal levels by hemagglutination (12–25 μg/ml, <1% normal). He has had occasional traumatic intramuscular hemorrhages, which usually do not require fibrinogen therapy. He last received fibrinogen in the form of cryoprecipitate 1 mo before this study. He had serum hepatitis, mumps, measles, and chicken pox as a child, but has no history of increased susceptibility to infections. He has had various allergic reactions, including urticaria (codeine, cryoprecipitate), wheezing (sulfonamides, cryoprecipitate), and hypotension (during intravenous pyelogram for ureterolithiasis).

J.G. is a 26-yr-old Caucasian male with congenital afibrinogenemia who has also been reported (8). His plasma does not form a visible clot and plasma fibrinogen has been undetectable. He has had numerous episodes of bleeding, beginning at birth and usually related to minor soft tissue trauma or dental problems. He has required intermittant fibrinogen replacement in the form of cryoprecipitate, the most recent transfusion 3 mo before this study. His other illnesses include Hbs Ag positive serum hepatitis (age 24) and a dental abscess (age 25). There is no history of increased susceptibility to infection and or allergic reactions.

Delayed-type skin tests. Intradermal skin tests were elicited on the outer aspect of the forearm as described (2). Antigens included purified protein derivative of tuberculin (PPD)[1]

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[1] *Abbreviations used in this paper*: PPD, purified protein derivative of tuberculin; SK-SD, streptokinase-streptodornase.

(250 U, Connaught Laboratories, Ltd., Willowdale, Ontario); mumps skin test antigen (2 colony-forming U, Lilly Laboratories); streptokinase-streptodornase (SK-SD) (25 U SK, 6.25 U SD, Massachusetts General Hospital Pharmacy); and monilia antigen (1 protein nitrogen U, Massachusetts General Hospital Pharmacy). Skin test sites were observed for immediate reactions and re-examined 40–48 h later. Erythema and induration were scored on a scale of 0 to +++ as described (2).

Biopsies from the SK-SD (C.M.) and monilia (J.G.) skin test sites were obtained at 40–48 h with a 2-mm biopsy punch after local anesthesia with xylocaine (2). Bleeding at the biopsy site was controlled by a pressure dressing. Tissue was bisected and one portion frozen for immunofluorescence. Frozen sections were stained with fluorescein conjugated anti-human fibrinogen, as well as with antibodies to immunoglobulin (Ig)G, IgA, IgM, IgD, IgE, C3, and albumin (N. L. Cappel Laboratories Inc., Cochranville, Pa.) by techniques described (2). The antifibrinogen antibodies had previously been absorbed with human serum conjugated to Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). Absorption with a fibrin clot removed all staining ability of the antifibrinogen. This antibody detects fibrinogen as well as fibrin and other larger fibrinogen fragments (2). The other portion of the biopsy was fixed in glutaraldehyde-paraformaldehyde and 1-μm Epon-embedded sections (Epon; Shell Chemical Co., Houston, Tex.) were prepared, and stained with Giemsa reagent (9). Infiltrating cells were quantitated per millimeter of skin surface with an optical micrometer on the full thickness of the biopsies using three separate sections as described (10).

Control intradermal skin tests were elicited in normal male volunteers with naturally acquired delayed hypersensitivity to microbial antigens. 23 positive delayed skin tests were biopsied at 48 h and studied by the same techniques. Three positive reactions from two normal individuals were biopsied and studied concurrently with those from the afibrinogenemic patients; the other biopsies have been reported (2, 9).

Written informed consent was obtained from all subjects before their participation in this study.

TABLE I

Delayed-Type Hypersensitivity Reactions in Congenital Afibrinogenemia and Normal Volunteers

Skin test	Mean diameter	Erythema	Induration	Fibrin*
	mm			
Afibrinogenemia‡				
SK-SD				
(C.M.)	32	++/+++	0	trace
(J.G.)	10	+	0	—
Monilia				
(C.M.)	28	++/+++	0	—
(J.G.)	34	+++	0	trace
Mumps				
(C.M.)	18	+	0	—
(J.G.)	21	++	0	—
PPD				
(C.M.)	0	0	0	—
(J.G.)	0	0	0	—
Normal§				
SK/SD (4)	22	++	++	++/+++
Monilia (8)	17	++	++	++/+++
Mumps (6)	16	++	++	++/+++
Old tuberculin (5)	18	++	++	++/+++

* Fibrin-fibrinogen detected by immunofluorescence. The dash indicates sites not biopsied.

‡ The results for the two subjects C.M. and J.G. are at 40 and 48 h, respectively.

§ Normal subjects were examined and biopsied 48 h after skin testing; number of skin tests in parentheses. Mean values are indicated. Three reactions, one each of SK-SD, monilia, and mumps, were studied at the same time as C.M. and J.G.; the others have been previously reported (2).

RESULTS

Delayed reactions in afibrinogenemic subjects. Both patients with congenital afibrinogenemia developed positive reactions to three of the antigens studied (SK-SD, monilia, and mumps) but not to PPD. No immediate reaction was detectable. The subjects noted that erythema developed about 6–12 h after testing and increased progressively in intensity and diameter up to the time of our formal evaluation at 40–48 h (Table I, Fig. 1A). The skin tests were not indurated and could not be distinguished from nearby normal skin by palpation. Even observation with tangential illumination revealed only slight diffuse swelling in the four more erythematous reactions. A trace of hemorrhage was present as a 1 to 2-mm discoloration at the injection site in three skin tests (SK-SD and monilia in C.M. and PPD in J.G.).

Immunofluorescence studies of the SK-SD reaction biopsy at 40 h (C.M.) demonstrated only traces of fibrinogen/fibrin in the form of granules and short fibrils in the upper reticular dermis (Fig. 1C). Similarly, in the monilia skin test biopsy at 48 h (J.G.), only traces of fibrin/fibrinogen were detectable, in the form of granular staining near one superficial dermal vessel. No diffuse fibrillar dermal fibrin deposition was found as is typical in normal subjects (see below). There was no significant staining for immunoglobulin, C3, or albumin in either the afibrinogenemic or normal subjects.

Light microscopy of the SK-SD and monilia reactions showed a prominent mononuclear infiltrate concentrated around venules of the superficial venous plexus (Fig. 1B). Small lymphocytes and lymphoblasts were numerous; monocytes/macrophages were less frequent. Quantitative analysis of the infiltrate revealed that the SK-SD reaction had 382±54 mononuclear cells/linear mm and 14±5 neutrophils/mm. The monilia reaction had 260±41 mononuclear cells/mm, 1±1 neutrophils/mm, and 2±1 basophils/mm. No eosinophils were detected. These correspond to the midrange of cellular infiltration we reported in normal subjects (9). The microvasculature showed striking alterations (3–4+) that included endothelial cell necrosis and hypertrophy (activation) as manifested by increased cytoplasm sufficient to encroach on the vascular lumen (11). The basement membrane enveloping these vessels also ex-

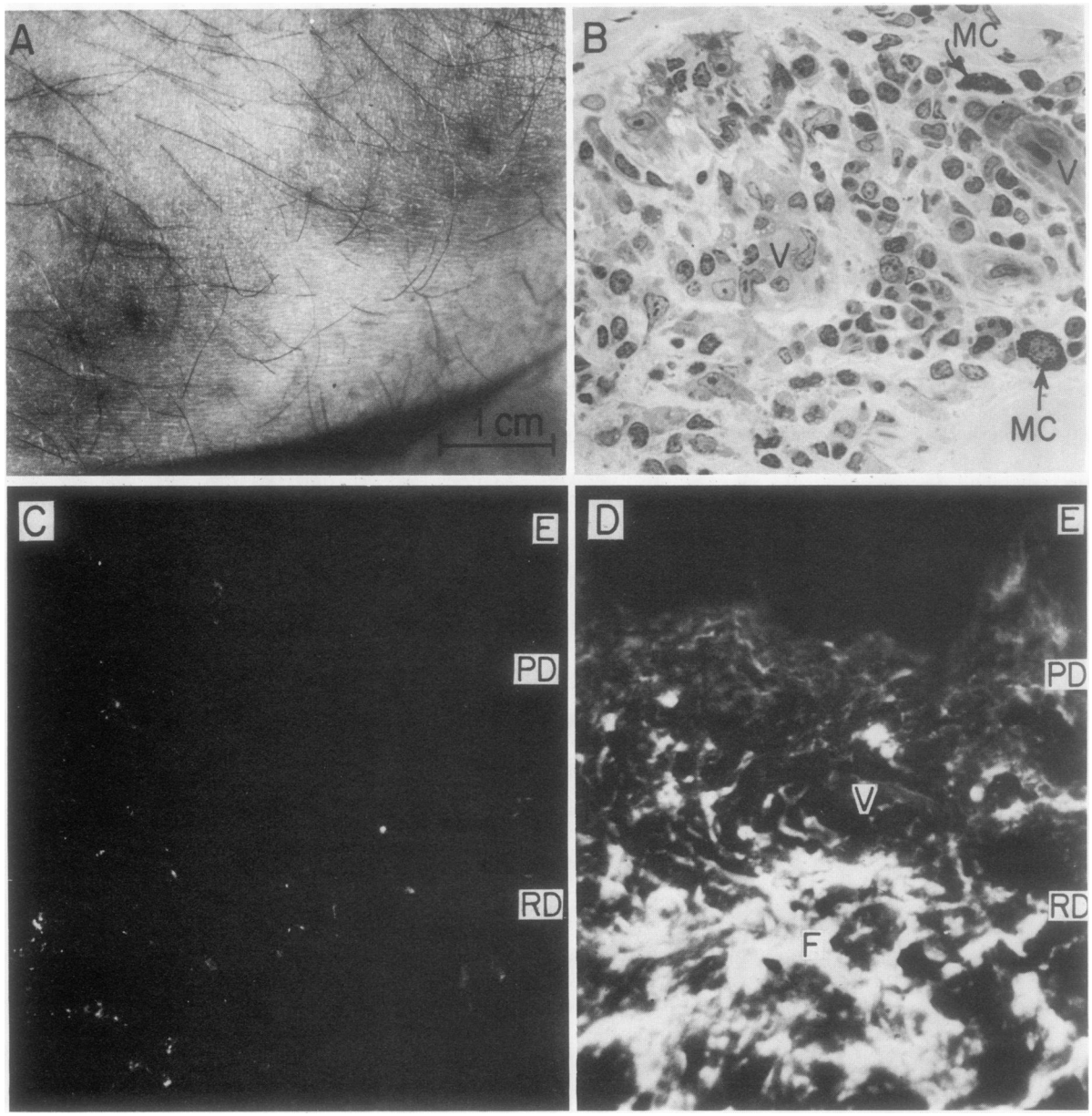

FIGURE 1 The SK-SD delayed reaction at 40 h in afibrinogenemic subject C.M. (A) intense erythema typical of strong delayed hypersensitivity is present in the SK-SD (left) and monilia (right) skin reactions. Focal hemorrhage is present in the injection site. (B) 1-μm Epon section of the SK-SD reactions shows a typical, intense perivascular mononuclear infiltrate present around reactive dermal vessels (V). Mast cells (MC) appear normal. Giemsa stain. ×500. (C) Immunofluorescence micrograph same SK-SD reaction stained with antifibrinogen. Only traces of fibrin/fibrinogen are detected as scattered fluorescent particles. The epidermis (E), papillary dermis (PD), and reticular dermis (RD) are indicated. ×120. (D) Immunofluorescence micrograph of 48 h delayed-type hypersensitivity reaction to monilia in a normal subject stained with antifibrinogen. Extensive, extravascular fibrin/fibrinogen (F) deposits are present in the dermis, a consistent feature of indurated delayed-type skin tests. Labeled as in C. ×120.

hibited swelling and thickening. Only rare erythrocyte extravasation was present.

Delayed reactions in normal subjects. All of 23 positive delayed skin tests in normal persons were erythematous and markedly indurated. Biopsies revealed extensive diffuse fibrillar fibrin/fibrinogen deposition in the

upper reticular dermis (Table I, Fig. 1D). The perivascular mononuclear infiltrate was similar to that in the afibrinogenemic patients and has been described (2, 9).

DISCUSSION

Two subjects with afibrinogenemia were found to have abnormal delayed type skin test reactions to several standard microbial antigens. While the kinetics, diameter, and intensity of erythema were typical of strong delayed hypersensitivity reactions, the induration that is characteristic of normal positive reactions failed to develop. In the two biopsy specimens studied, a typical perivascular mononuclear infiltrate was present but only traces of fibrin/fibrinogen were detectable by immunofluorescence. In contrast, indurated delayed reactions in normal subjects, without exception, had extensive deposits of fibrin/fibrinogen (2, 9, 10).

We have hypothesized that fibrin deposition is responsible for the induration that is a hallmark of classic delayed-type skin reactions for several reasons. Extensive fibrin deposition occurs in classic delayed reactions to all antigens tested in guinea pigs (12) and man (2, 10). Anticoagulation with warfarin or heparin inhibits induration (12, 13–15), and in parallel, warfarin inhibits ^{125}I-fibrinogen accumulation in skin tests in guinea pigs (12). Intradermal injection of fibrinogen, but not fibrin degradation products or other plasma proteins, causes induration in guinea pig skin (unpublished data). Other agents known to interfere with the clotting system at various stages also inhibit induration (16–18). However, the mechanism of the drug-induced inhibition has been unclear, because these agents may have diverse effects.

The present study in subjects with a genetic defect in fibrinogen synthesis provides specific evidence that it is fibrin or fibrinogen that is necessary for the development of induration. Delayed-type hypersensitivity reactions in the two afibrinogenemic subjects lacked induration and prominent fibrin deposits, but were otherwise typical in their time-course, erythema, and mononuclear cell infiltrate. Certain components of delayed skin test reactions therefore apparently do not require circulating fibrinogen or fibrin deposition, or require only extremely low levels. These include emigration of lymphocytes, local lymphoblast transformation, and the profound endothelial activation/necrosis and basement membrane thickening of the small dermal vessels cuffed by these cells. Fibrin deposits might be expected to exert some influence on the cellular infiltrate because macrophages have surface receptors for fibrin/fibrinogen (19, 20), but recognizable macrophages are uncommon in delayed reactions in man (9) and alterations in these cells would be difficult to evaluate.

Little is known about the pathogenesis of the fibrin deposition in cell-mediated immunologic reactions. At least two factors seem important. Most likely increased vascular permeability with fibrinogen extravasation is a necessary first step (12). Thromboplastins, perhaps from activated mononuclear cells, may then promote the conversion of extravasated fibrinogen to fibrin (15, 21). The result is an extravascular fibrin gel, which is believed to be the basis of the induration.

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