0022-202X/85/8501s-0129s$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 85:129s–132s, 1985

Vol. 85, No. 1 Supplement
Printed in U.S.A.

A Study of Human Serum Sickness

THOMAS J. LAWLEY, M.D., LEONARD BIELORY, M.D., PEDRO GASCON, M.D., PH.D., KIM B. YANCEY, M.D., NEAL S. YOUNG, M.D., AND MICHAEL M. FRANK, M.D.

Dermatology Branch, National Cancer Institute (TJL, KBY), Clinical Hematology Branch, National Heart, Lung, and Blood Institute (LB, PG, NSY), and Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases (MMF), National Institutes of Health, Bethesda, Maryland, U.S.A.

Twelve patients with bone marrow failure, who were undergoing therapy with daily intravenous infusions of horse antithymocyte globulin, were studied for the development of serum sickness. Eleven of 12 patients developed typical signs and symptoms of serum sickness 8–13 days after the initiation of treatment. These included fever, malaise, cutaneous eruptions, arthralgias, gastrointestinal disturbances, and lymphadenopathy. Eleven of 12 patients developed high levels of circulating immune complexes during serum sickness. All 12 patients also had concomitant decreases of serum C3 and C4 levels. In addition to urticarial and/or morbilliform eruptions, 8 of 11 patients also developed a serpiginous band of erythema along the sides of the fingers, hands, toes, or feet as an early cutaneous sign of serum sickness. Direct immunofluorescence of lesional skin biopsies during serum sickness revealed deposits of immunoglobulin or complement in the walls of small cutaneous blood vessels in 3 of 5 patients. These findings indicate that circulating immune complexes play a central role in the pathophysiology of human serum sickness.

The first detailed description of human serum sickness was presented by Clemens von Pirquet and Bela Schick in their classic monograph written in 1905 entitled "Die Serumkrankheit" or "Serum Sickness" [1]. In the early 1900's these 2 physicians were attempting to use serum therapy to control various infectious diseases. Serum therapy consisted of the injection of hyperimmune heterologous serum into a human subject suffering from a particular disease. In their cases von Pirquet and Schick were using horse antiserum to diphtheria organisms to treat diphtheria infections in children. von Pirquet and Schick noted that s.c. injections of horse serum produced a reproducible reaction pattern in many of their patients. This consisted of fever, malaise, cutaneous eruptions, lymphadenopathy, arthralgias, leukopenia, and proteinuria which began 8–12 days after injection of the horse serum. They also noted that the incidence of serum sickness was related to the amount of horse serum administered. Individuals who received 5–10 cc s.c. had an incidence of serum sickness of 5–10% while those who received large amounts of horse serum (up to 200 cc) had an incidence of serum sickness approaching 90%. Although von Pirquet and Schick suspected that serum sickness was a manifestation of an immune reaction of the host to the foreign antigens injected, the methodology needed to pursue this line of inquiry was not available.

Later, several large retrospective clinical studies of serum sickness confirmed the findings of von Pirquet and Schick [2–4]. However, it was not until the studies of Germuth et al and Dixon et al, using animal models of serum sickness, that insight into the pathophysiology of this disease was achieved [5–7]. These investigators found that foreign proteins injected into their animals resulted in a strong antibody response, and that the host antibody combined with the injected antigen to form circulating antigen-antibody complexes. These immune complexes were capable of activating complement as well as causing tissue damage such as carditis, arteritis, and glomerulonephritis. These findings clearly indicated that circulating immune complexes mediated serum sickness in animals and by inference suggested that the same was true in humans. Since that time, numerous single case reports of serum sickness have been published but few patients have undergone an in-depth immunological analysis [8–11].

In this study we present a prospective clinical and immunological study of serum sickness occurring in patients with bone marrow failure treated with infusions of horse antithymocyte globulin (ATG) [12].

MATERIALS AND METHODS

Patients

Twelve patients were admitted to the Clinical Hematology Branch of the National Institutes of Health. All patients had been diagnosed as having bone marrow failure. After obtaining informed consent, the patients were entered into a protocol using i.v. infusions of horse antithymocyte globulin (ATGAM, Upjohn Co., Kalamazoo, Michigan) for the treatment of bone marrow failure. The i.v. infusions were given over 5 h at a dosage of 15 mg/kg/day for 10 days. The patients also received methylprednisolone 1 mg/kg/day which was increased to 1.5 mg/kg/day during serum sickness. Blood samples were obtained before and after ATG therapy as well as daily throughout its course.

Immune Complex and Complement Assays

The ^{125}I-C1q binding assay was performed as previously described [13]. The upper limit of normal in the C1q-binding assay is 10%. The Raji cell assay for IgG-containing circulating immune complexes was performed according to the method of Theofilopoulos [14] as modified by Hall [15].

Assays for hemolytic C3 and C4 titers were performed as previously described [16,17].

Plasma levels of C3a/C3a des Arg were measured by a commercially available radioimmunoassay (Upjohn Diagnostics, Kalamazoo, Michigan). The range of normals in our laboratory is 90–880 ng/ml.

Direct Immunofluorescence

Three-millimeter skin punch biopsies of early (< 24 h) lesional skin were obtained in 5 patients and processed for routine direct immunofluorescence. Four-micrometer cryostat sections were then stained with FITC-conjugated goat antihuman IgG (1:80), IgA (1:20), IgM (1:20), IgE (1:20), C3 (1:20), and fibrin (1:20) (Tago, Inc., Burlingame, California). Specimens were examined with a Leitz epifluorescence microscope.

Histopathology

Three-millimeter skin punch biopsies of cutaneous eruptions occurring during serum sickness were obtained in 5 patients, fixed in 10% neutral formalin, and processed for routine light microscopy.

Reprint requests to: Thomas J. Lawley, M.D., Dermatology Branch, Building 10, Room 12N238, National Institutes of Health, Bethesda, Maryland 20205.

Abbreviations:
ATG: antithymocyte globulin
BSA: bovine serum albumin

RESULTS

Clinical Observations

All 12 patients completed the 10-day course of ATG therapy. Eleven of 12 patients developed the signs and symptoms of serum sickness 8–13 days after beginning ATG. These included fever, malaise, cutaneous eruptions, arthralgias, gastrointestinal disturbances, and lymphadenopathy (Table I).

A variety of cutaneous eruptions were documented in these patients during ATG therapy. Eight patients developed an urticarial reaction to the ATG infusions very early (day 1–3) in their treatment course. This eruption responded to therapy with antihistamines and was clearly not a manifestation of serum sickness. When serum sickness did occur in these patients, it was always associated with cutaneous eruptions. The onset of cutaneous eruptions associated with serum sickness was closely related in time to the onset of the other manifestations. The serum sickness related eruptions began on day 9.5 ± 2.6. Five patients had only morbilliform eruptions which began on the trunk as erythematous patches before spreading to involve the extemities. Five patients had both morbilliform and urticarial eruptions during serum sickness and 1 patient had urticaria alone.

In addition to the urticarial and morbilliform eruptions, 8 patients also manifested an unusual cutaneous sign during serum sickness. This consisted of a serpiginous band of erythema along the sides of the fingers, hands, toes, and feet at the margin of palmar or plantar skin. The erythema was quite subtle, at first, but became more obvious as serum sickness progressed. In many patients, because of their marked thrombocytopenia, the erythema was replaced by purpura. This band of erythema was in many instances the earliest cutaneous manifestation of serum sickness (Fig 1).

Circulating Immune Complexes

Elevated levels of circulating immune complexes were found in 11 of 12 patients treated with ATG, as detected by the C1q binding assay. The rise and fall of immune complexes were closely correlated with the overall time course of serum sickness (Fig 2). The mean of pretreatment values of C1q binding activity for the 12 patients was 12.4 ± 7.2%. During serum sickness the mean peak C1q binding level reached 47.2 ± 13.5% on day 12.3 ± 2.3. A rise and fall of immune complexes were also detected using the Raji cell assay. In the seven patients studied, the mean baseline immune complex level was 0.94 ± 0.29. This rose to a mean peak level of 1.47 ± 0.39 on day 10.1 ± 2.3.

TABLE I. *Clinical signs and symptoms in 11 patients with serum sickness*

Fever	100%
Malaise	100%
Cutaneous eruptions	100%
Arthralgias	55%
GI disturbances	45%
Lymphadenopathy	18%

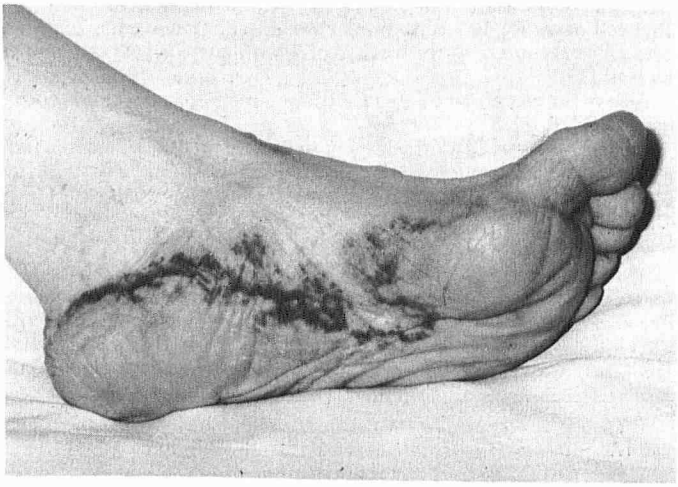

FIG 1. The foot of a patient with serum sickness showing hemorrhage in a serpiginous band at the border of plantar skin. The hemorrhage which occurred because of this patient's marked thrombocytopenia was preceded by erythema in exactly the same distribution.

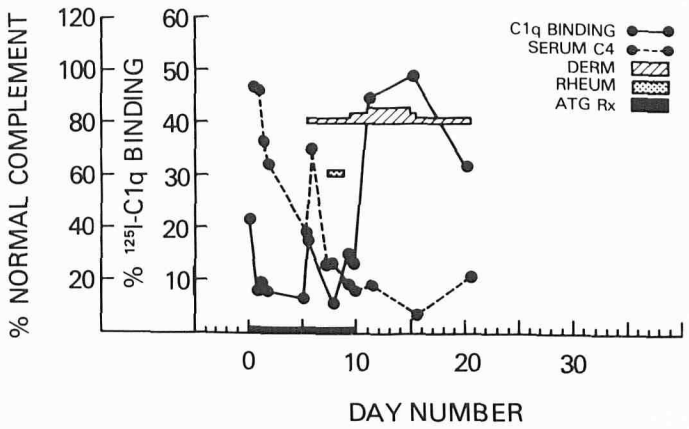

FIG 2. Serum immune complexes, complement levels, and symptoms in a patient with serum sickness. A graph showing the time course of changes in C1q binding activity and C4 levels as well as the occurrence of dermatologic and rheumatologic disease in a representative patient with ATG-induced serum sickness. Variations in height of bar graph showing dermatologic disease represent changes in disease severity.

TABLE II. *Serum C3 profiles in patients treated with ATG*

Patient	Baseline C3 concentration (% of control)	Nadir C3 concentration (% of control)	Nadir day	% Decrease in C3
1	114%	10%	16	91%
2	103%	15%	15	85%
3	24%	0	9	100%
4	94%	31%	10	67%
5	28%	20%	9	29%
6	68%	38%	11	44%
7	115%	63%	11	45%
8	53%	0	10	100%
9	65%	43%	4	34%
10	21%	0	8	100%
11	68%	18%	15	74%
12	100%	11%	9	89%
Mean	71%	20.8%	10.6	71.5%
SD	± 34.5	± 19.6	± 3.4	± 27.0

Complement Levels

Serum C3 was measured in all patients (Table II). Five patients began ATG therapy with normal C3 levels (> 90% of control) and 7 patients had decreased baseline C3 levels (< 90% of control). All 12 patients showed decreased C3 levels during serum sickness. The time course of C3 decreases closely paralleled the development of the signs and symptoms of serum sickness and the appearance of circulating immune complexes. C3 decreases ranged from 29–100% of baseline levels and reached nadir values at day 10.6 ± 3.4. The mean decrease in C3 levels was 71.5 ± 27% (Table II).

Serum C4 levels were also markedly depressed in all 12 patients during serum sickness (Fig 2). The mean baseline C4 level was 77.8 ± 36.8%. The mean nadir level of serum C4 during serum sickness was 6.6 ± 8.5% which occurred on day

TABLE III. *Serum C4 profiles in patients treated with ATG*

Patient	Baseline C4 concentration (% of control)	Nadir C4 concentration (% of control)	Nadir Day	% Decrease in C4
1	62%	14%	16	77%
2	54%	4%	13	93%
3	132%	0	9	100%
4	20%	1%	10	95%
5	31%	25%	9	19%
6	94%	10%	11	89%
7	65%	19%	7	71%
8	60%	0	10	100%
9	105%	3%	4	97%
10	72%	0	8	100%
11	138%	3%	15	98%
12	100%	0	9	100%
Mean	77.8%	6.6%	10.0	86.6%
SD	± 36.8	± 8.5	± 3.3	± 23.3

10 ± 3.3. The mean decrease of serum C4 was 86.6 ± 23.3% (Table III). Again the time course of C4 reductions correlated well with the onset of clinical disease and the appearance of circulating immune complexes.

Plasma levels of C3a/C3a des Arg were measured serially in 4 patients. C3a/C3a des Arg is a cleavage fragment of C3 and is a potent anaphylatoxin. All 4 patients showed increases in plasma C3a levels during serum sickness. The mean pretreatment baseline value of 11.5 ± 13.7 μg/ml increased to a mean peak level of 42.8 ± 30.7 μg/ml on day 9.5 ± 5.3.

Direct Immunofluorescence

Skin biopsies of cutaneous lesions less than 24-h old were obtained in 5 patients. Of the 5 biopsies obtained during serum sickness, 3 revealed immunoreactants in the walls of superficial cutaneous blood vessels. IgM deposits were found in 3 patients, IgE and C3 in 2, and IgA in one. No deposits of IgG were found in any patients.

Histopathology

Skin biopsies were obtained from 5 patients during serum sickness. All patients were neutropenic and thrombocytopenic. The biopsies showed only low grade perivascular infiltrates of lymphocytes and histiocytes associated in some cases with modest stromal edema. There was no evidence of significant vascular damage.

DISCUSSION

The constellation of signs and symptoms that clinically define the syndrome of serum sickness were described in great detail by von Pirquet and Schick at the turn of the century, but the pathophysiology of serum sickness remained speculative for many years. The development of animal models of serum sickness in the 1950's and 1960's provided hard evidence that not only was serum sickness immunologically mediated, but that circulating immune complexes played a central role in its pathophysiology. In one such model, immunized rabbits were infused with radiolabeled bovine serum albumin (BSA) and the animals were monitored clinically and immunologically over time [7]. After undergoing initial equilibration with the extravascular space, the serum concentration of the BSA gradually decreased through degradative processes. However, on about day 8 after the single injection of antigen, the levels of free BSA declined precipitously. At this same time immune complexes appeared in the circulation, complement levels decreased, and the animals became ill. Antigen, antibody, and complement were identified at sites of tissue injury including the kidney and blood vessels. These findings indicated that circulating immune complexes were important in the pathophysiology of this animal model of serum sickness and suggested that the same was true for human serum sickness. Although a number of case reports have appeared in which immunologic parameters have been examined, there has been no large scale, prospective, detailed study of the clinical and immunological events in human serum sickness until recently [12].

Our patients were infused with large amounts of horse IgG ranging approximately from 750 mg to 1.5 g per day for 10 days. Eleven of 12 patients developed typical clinical manifestations of serum sickness and did so in a time course remarkably similar to that described by von Pirquet and Schick. The serum sickness was self-limited and usually resolved several days after ATG theapy ended.

The cutaneous manifestations of serum sickness were of special interest. They consisted mainly of morbilliform eruptions either alone or in conjunction with urticaria. Additionally a high percentage of these individuals developed an unusual, previously unreported eruption along the sides of the fingers, hands, toes, and feet at the margin of palmar or plantar skin. This eruption began as a subtle serpiginous band of erythema and progressed in these patients to become purpuric, presumably secondary to their thrombocytopenia. The eruption in many instances was the earliest cutaneous sign of serum sickness. It appears to be a useful cutaneous marker for ATG-induced serum sickness in particular , and we suspect for serum sickness in general.

The finding that these patients developed large rises and falls in the levels of circulating immune complexes that paralleled the clinical manifestations of serum sickness suggests that these immune complexes cause clinical disease. This is further buttressed by the parallel decreases in serum C3 and C4 levels during serum sickness presumably caused by activation of the complement system by the circulating immune complexes. It was also interesting to find very high plasma levels of C3a. C3a is a potent anaphylatoxin capable of inducing urticarial lesions when introduced into human skin. The high levels found during serum sickness suggest the C3a may play an important role in the cutaneous manifestations of serum sickness. Finally, the detection of deposits of immunoglobulin and complement in the walls of small cutaneous blood vessels in a majority of the patients tested is further evidence that circulating immune complexes also mediate the cutaneous manifestations of serum sickness.

All of these patients were receiving substantial amounts of systemic glucocorticoids before and during serum sickness, as part of the investigative protocol. In spite of this, almost all of the patients developed serum sickness. This indicates that methylprednisolone in doses of 1 mg/kg/day did not prevent the development of serum sickness. It is possible, however, that the glucocorticoid therapy did decrease the severity of the illness. It is also possible that these patients' underlying illness, bone marrow failure, and associated leukopenia and thrombocytopenia, may have also affected their clinical course.

These data provide the first prospective clinical and immunological analysis of serum sickness in man. They confirm in man the immunological observations made in animal models of the disease and present a previously undescribed cutaneous sign of serum sickness.

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