

FLUORESCENT ANTIGLOBULIN STUDIES IN LEUKOPENIC AND RELATED DISORDERS *

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Increasing interest in the search for antileukocyte factors in clinical syndromes characterized by leukopenia has been evident in recent years (1-6). The demonstration of what appear to be auto-antibodies in idiopathic thrombocytopenic purpura (7-9) and acquired hemolytic anemia (10-12) has, by analogy, directed attention to the possibility of a similar mechanism operating in certain leukopenic conditions (1-6, 13). Similarly, the effects of drug sensitivity have been recognized to affect any or all of the three formed elements of the peripheral blood (14-20). Evidence has also been presented that in patients requiring multiple transfusions, powerful leukocyte antibodies may be produced and give rise to undue reactions (21-23). Finally, the reported occurrence of anti-leukocyte substances in patients with aleukemic leukemia and Hodgkin's disease presents another area for further investigation (2, 24-27).

The lack of a simple, reproducible, reliable technique for the detection of leukocyte antibodies still constitutes a limiting factor in this branch of immunohematology (3, 25, 28, 29). With the development of fluorescein-labeled antibodies (30), a new tool has become available for the study of immune reactions. This paper deals with the application of this method in the search for anti-leukocyte factors in several disorders, with special emphasis on rheumatoid arthritis, Felty's syndrome and systemic lupus erythematosus (SLE). A preliminary report has been presented (31).

MATERIALS AND METHODS

Blood smears. Capillary blood from normal individuals of varying ABO and Rh blood types was used. Smears

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prepared by the slide method were immediately fixed in 95 per cent ethanol for 45 minutes, rinsed in distilled water and dried before using.

Human sera. Sera from 112 individuals were obtained. When not tested within 24 hours from the time they were drawn, they were stored at -20° C. until used. Twenty laboratory workers served as normal controls. All the patients studied were examined by at least one of

TABLE I
Results of test on sera of 112 individuals studied

Clinical diagnosis	Total no. of cases	Nuclear leukocyte fluorescence	
		Positive	Negative
Systemic lupus erythematosus	15	15	0
Rheumatoid arthritis			
Felty's syndrome	10	10	0
Uncomplicated	13	2	11
Periarteritis nodosa	1	0	1
Serum sickness	1	0	1
Erythema nodosum	1	0	1
Rheumatic fever and chorea	3	0	3
Discoid lupus	2	0	2
Hyperglobulinemia			
Laennec's cirrhosis	3	0	3
Bronchogenic carcinoma	1	0	1
Multiple myeloma	3	0	3
Asthma (on steroids for 3 years)	3	0	3
Multiple transfusions	4	0	4
Idiopathic thrombocytopenic purpura	5	0	5
Granulocytic leukemia			
Aleukemic	3	0	3
Leukemic	7	0	7
Lymphocytic leukemia			
Aleukemic	3	0	3
Leukemic	2	0	2
Polycythemia vera	8	0	8
Hodgkin's disease	4	0	4
Normal laboratory personnel	20	0	20
Totals	112	27	85

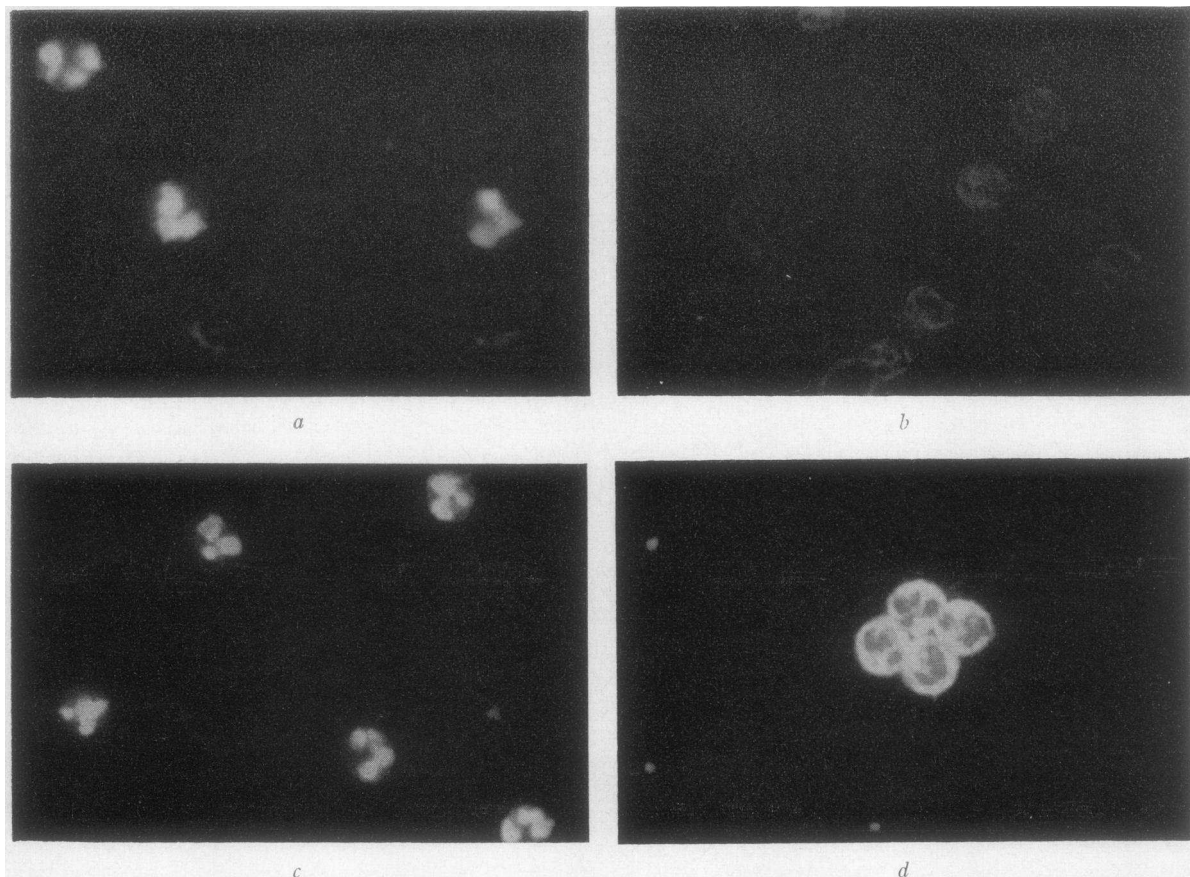


FIG. 1. PHOTOMICROGRAPHS OF POLYMORPHONUCLEAR LEUKOCYTES FROM NORMAL BLOOD SMEARS TREATED WITH HUMAN SERA AND SUPERIMPOSED WITH FLUORESC EIN-LABELED RABBIT ANT HUMAN GLOBULIN (COOMBS') SERUM

Human sera from: *a*) systemic lupus erythematusus; *b*) normal control; *c*) Felty's syndrome; *d*) normal control exposed to antiglobulin *not* absorbed with rabbit bone marrow. Note nonspecific cytoplasmic fluorescence.

us, except where otherwise indicated. The different disease states investigated are listed in Table I.

Preparation of fluorescent antihuman globulin (Coombs') serum. Human γ -globulin (Cohn Fraction II) in Freund's adjuvant (32) was injected intramuscularly into 3 Kg. male albino rabbits. The dosage schedule was 50 mg. of γ -globulin on four successive days of each week for four weeks with a repeat series after a two week interval. The animals were sacrificed seven days following the last injection. The sera with precipitin titers varying from 1:40,000 to 1:80,000 were pooled and stored at -20°C .

The fluorescein¹ conjugate was prepared by the method described by Coons and Kaplan (30) with the following modification. The rabbit serum was precipitated twice with a half saturated ammonium sulfate solution, resuspended in 0.15 M NaCl and dialyzed against a solu-

tion of 0.15 M NaCl containing 0.01 M phosphate until no sulfate was detectable (usually four to six days) before conjugation with fluorescein isocyanate. The precipitation and dialysis were carried out at 4°C .

The labeled conjugate was stored in 5 to 10 ml. aliquots at -20°C . and was absorbed twice with acetone-dried rabbit bone marrow (33, 34) before use.

Testing procedure. A few drops of the human serum to be tested were placed on a thin portion of the slide in direct contact with the fixed normal blood smear and allowed to stand at room temperature in a moist chamber for one hour. The slide was then washed in running tap water for at least 30 minutes and air dried. The labeled rabbit antihuman globulin serum (Coombs' serum) was then superimposed on the test area of the smear for one hour under the same conditions. Vigorous washing for one hour or more was necessary to remove nonspecific labeling, and an even clearer differentiation was obtained if the preparations were allowed to stand 24 hours before making the observations by ultraviolet microscopy.

¹ The fluorescein amine was kindly supplied by Dr. Leland Pence, of Difco Laboratories.

Readings. All slides were read "blind" by each one of the authors. Early in the study, as many as five separate readings were done by each observer on different days, and some sera were tested on multiple occasions against smears from six or more individuals of different blood groups. It soon became apparent, however, that consistent results were being regularly obtained, and as our experience increased, two readings apiece were considered sufficient. For the sake of uniformity, only smears from O Rh-negative normals were used in the latter part of the study, although definite, naturally occurring leukocyte isoantibodies could not be demonstrated and no specific relationship to erythrocyte antigens was observed (1, 23, 35-37). An important aspect of the interpretation rests upon having a known positive serum and a known negative serum included in each series of slides stained. The presence or absence of the apple-green (specific) fluorescence constituted a positive or negative reading.

Microscopy and photographic equipment. A Leitz Ortholux microscope with a cardioid dark field condenser was used, with a 150 watt Philips mercury vapor lamp providing the ultraviolet light source. Thirty-five mm. daylight super Anscochrome® film was used for recording the slides.

Lupus erythematosus (L.E.) cell preparations and sheep cell hemagglutination titers. L.E. cell preparations were performed by the method of Magath and Winkle (38). The sheep cell hemagglutination titers were carried out as described by Craig, Kerby and Persons (39).

In vivo studies. Whole blood was collected in vacuum bottles containing acid citrate dextrose (ACD) solution and, after centrifuging, the plasma was transferred to vacuum containers under aseptic conditions and stored at -20° C. until ready for use. The recipients were ABO and Rh-compatible patients with far advanced neoplasms not obviously involving the bone marrow and with normal peripheral leukocyte counts. In each case the recipient was given first the plasma from a leukopenic subject and, several days later, a similar amount of normal plasma under identical conditions.

RESULTS

Nonspecificity of unabsorbed fluorescent antihuman globulin (Coombs') serum

The use of labeled antihuman globulin serum which had not been absorbed with acetone-dried rabbit bone marrow caused cytoplasmic fluorescence of leukocytes previously exposed to normal serum (Figure 1d). This made the differentiation of positive and negative smears extremely difficult or impossible. After absorption with rabbit bone marrow, however, this nonspecific cytoplasmic labeling diminished markedly (Figure 1b), allowing recognition of unequivocal differ-

ences between cell nuclei coated with positive and negative sera (Figures 1a, 1b, and 1c).

Specificity of absorbed antihuman globulin (Coombs') serum

To demonstrate the specificity of the labeled conjugate for human globulin, nonfluorescent rabbit antihuman globulin serum was superimposed on blood smears previously exposed to known positive sera from patients with SLE or Felty's syndrome. After subsequent exposure to labeled conjugate, the nuclei showed no fluorescence.

Systemic lupus erythematosus, rheumatoid arthritis and allied disorders

Fifteen patients with SLE were studied (Table II). In each case a strongly positive nuclear fluorescence was obtained by the technique described (Figure 1a). No difference was observed when smears of several individuals of various blood groups were used. All except one had positive L.E. cell tests at the time the fluorescein test was performed. One patient (C.S.) was of special interest because of a positive nuclear fluorescence reading with absence of demonstrable L.E. cells. Her clinical picture was quite compatible with SLE and on subsequent examination, two months later, several positive L.E. preparations were obtained with her serum.

The sera of 23 patients with the clinical picture of rheumatoid arthritis were obtained (Table II). In 10 of these a clinical diagnosis of Felty's syndrome was made and, for the purposes of the discussion, this group will be considered separately. In general this "label" was used when the classic triad of leukopenia, splenomegaly and rheumatoid arthritis was present without serological or other clinical manifestations of SLE. The following exceptions or variations to this picture were present. Four patients with Felty's syndrome were studied post splenectomy and, though previously leukopenic, their total leukocyte counts were normal at this time. Splenomegaly was, or had been, present in all but two cases; one of these (I.B.) was the only patient in this group with a positive L.E. cell test. She had a documented history of rheumatoid arthritis for 26 years with classical, severe joint changes, and at no time had any evidence of serositis, nephritis, carditis or

TABLE II
Patients with systemic lupus erythematosus, Felty's syndrome and rheumatoid arthritis

Patient	Age and sex	Clinical diagnosis	Splenomegaly	WBC $\times 10^3$	Serum proteins A/G	Sheep cell titers	L.E. prep.	Nuclear fluor.
					Gm./100 ml.			
1. A. T.	39F	SLE	Not Palp.	5.8	3.4/5.5	1:20	+	+
2. S. C.	31F	SLE	Not Palp.	5.0	4.5/3.4	Neg.	+	+
3. C. S.	52F	SLE	Not Palp.	3.8	4.3/5.5	1:320	Neg.*	+
4. H. N.	33F	SLE	Not Palp.	5.8	3.0/4.4	1:200	+	+
5. I. L.	58F	SLE	Not Palp.	3.3	2.6/3.4	1:1600	+	+
6. B. M.	15F	SLE	Not Palp.	5.4	1.9/5.6	Neg.	+	+
7. L. T.	46F	SLE	Not Palp.	5.5	4.5/3.6	Neg.	+	+
8. D. Mi.	22F	SLE	Not Palp.	6.0	4.3/3.6	1:80	+	+
9. D. Me.	32F	SLE	Not Palp.	14.0	3.4/5.0	Neg.	+	+
10. R. D.	44F	SLE	Not Palp.	3.1	2.7/3.9		+	+
11. J. D.	18F	SLE	Not Palp.	3.4	1.2/2.3		+	+
12. M. G.	15F	SLE	Not Palp.	4.5	3.1/2.3	1:20	+	+
13. D. B.	31M	SLE	3 cm.	7.1	3.8/5.3	1:20	+	+
14. G. N.	62M	SLE	Not Palp.	4.9	2.8/4.6	1:80	+	+
15. B. V.	25F	SLE	Not Palp.	7.5	4.3/4.3	1:20	+	+
1. I. B.	59F	Felty's	Not Palp.	3.0	3.0/3.2	1:400	+	+
2. R. S.	65M	Felty's	10 cm.	1.0	2.8/4.1	1:80	Neg.	+
3. B. H.	79F	Felty's	4 cm.	1.2	3.7/3.9	Neg.	Neg.	+
4. E. B.	66M	Felty's	Not Palp.	2.8	4.7/3.4	1:1280	Neg.	+
5. K. F.	70F	Felty's	By X-ray	0.8	3.2/4.0	1:80	Neg.	+
6. H. L.†	55F	Felty's	4 cm.	2.2		1:320	Neg.	+
7. H. C.	47F	Felty's	10 cm. (1958)‡	0.8 6.6§	2.8/3.0 2.6/1.9§	1:5120 1:2560§	Neg. Neg.§	+
8. L. M.	58F	Felty's	3 cm. (1953)‡	1.6 6.6§	3.6/4.8 3.7/3.7§	1:640§	Neg.§	+
9. T. Mc.†	65M	Felty's	5 cm. (1958)‡	1.7 8.9§	3.9/3.9	1:160§	Neg. Neg.§	+
10. L. Mc.	61F	Felty's	3 cm. (1953)‡	1.4 5.6§	5.1/3.3 4.5/3.1§	1:40§	Neg.§	+
1. L. B.	54F	Rh. Arth.	Not Palp.	5.0	3.7/3.0	1:320	Neg.	+
2. H. W.	33M	Rh. Arth.	Not Palp.	6.8	4.7/3.4	1:1280	Neg.	+
3. C. V.	35M	Rh. Arth.	Not Palp.	10.0	4.3/2.1	1:2560	Neg.	Neg.
4. C. D.	54M	Rh. Arth.	Not Palp.	7.8	4.1/2.6	1:1500	Neg.	Neg.
5. A. J.	71M	Rh. Arth.	Not Palp.	8.5	4.0/3.6	1:160	Neg.	Neg.
6. L. S.	27F	Rh. Arth.	Not Palp.	8.5	3.9/2.3	1:40	Neg.	Neg.
7. M. J.	57F	Rh. Arth.	Not Palp.	6.5	2.6/3.0	1:320	Neg.	Neg.
8. W. M.	60M	Rh. Arth.	Not Palp.	7.4	4.7/2.2	1:20		Neg.
9. C. Mc.	55M	Rh. Arth.	Not Palp.	7.8	4.2/2.4		Neg.	Neg.
10. H. S.	50F	Rh. Arth.	Not Palp.	8.5	4.9/2.2	1:320	Neg.	Neg.
11. M. D.	56F	Rh. Arth.	Not Palp.	12.0		1:20	Neg.	Neg.
12. H. B.	66M	Rh. Arth.	Not Palp.	11.5		Neg.	Neg.	Neg.
13. G. H.	65M	Rh. Arth.	Not Palp.	6.3	4.1/2.6	1:1280	Neg.	Neg.

* Negative at time of positive fluorescent antiglobulin test. Two months later positive L.E. preps.

† We are grateful to Dr. W. C. Moloney, Boston, Mass. for allowing us to study this patient.

‡ Date of splenectomy.

§ Postsplenectomy determinations.

Blank spaces, no determination performed.

dermatitis. Hemolytic anemia and thrombocytopenia were absent and the serological test for syphilis (VDRL) was negative. She had received infrequent small doses of steroids intermittently since 1954.

The sera from each of these 10 patients produced strong nuclear fluorescence which resembled in all respects the response obtained with the sera from

the patients with SLE (Figures 1a and 1c). The presence of positive nuclear fluorescence tests in four patients (L.M., L.Mc., T.Mc. and H.C.) for periods up to five years after splenectomy, in the presence of normal leukocyte counts, is of particular interest.

Of the remaining 13 patients with uncomplicated rheumatoid arthritis, the sera from two

contained a factor which caused nuclear fluorescence. None of these patients had positive L.E. cell tests.

A group of eight patients with other forms of possibly related disorders, all without splenomegaly or leukopenia and with negative L.E. cell tests, showed negative results with the fluorescent staining technique (Table I).

Nonrheumatic patients with hyperglobulinemia or on steroid therapy

To test the possibility that hyperglobulinemia *per se* may produce a positive reaction, the sera of seven patients with elevated globulins were tested. Three of these had Laennec's cirrhosis with globulins ranging from 3.0 to 3.8 Gm. per 100 ml. Three had multiple myeloma with globulins of 5.9, 11.2 and 11.3 Gm. per 100 ml., respectively, and one patient with bronchogenic carcinoma had a globulin of 4.6 Gm. per 100 ml. The fluorescent antiglobulin test was negative in all.

To exclude a possible effect of steroid therapy on the mechanism of the test, the sera of three patients with chronic bronchial asthma were tested. All had been receiving significant doses of steroids for at least three years and all showed negative results.

Patients receiving multiple transfusions

The sera from four patients who had received multiple transfusions were studied. Three of these patients had received 80, 97 and 232 transfusions, respectively, and two of them experienced repeated, nonhemolytic, febrile reactions. The serum from the fourth patient was known to contain a powerful leukoagglutinin.² A noticeable increase of leukocyte fluorescence was observed in all. The location of this was at first interpreted to be nuclear (31), but with the greater magnification and better resolution of the microscopic equipment now available to us, it is clear that all the fluorescence is confined to the cytoplasm.

Idiopathic thrombocytopenic purpura

Because it is known that destructive processes of the three formed elements of the peripheral

blood may on occasions show multiple involvement (1, 4, 13, 40), sera from five patients with idiopathic thrombocytopenic purpura were studied for evidence of antileukocyte globulins. Leukocyte fluorescence was negative in all cases, but particularly striking platelet fluorescence was observed in one case. This suggests the possibility of developing a similar fluorescent antiglobulin test for platelets.

Leukemias, polycythemia vera and Hodgkin's disease

A total of 27 patients with these disorders were studied, including six cases with aleukemic leukemia (Table I). In none was the presence of antileukocyte factors demonstrated by the present technique.

In vivo studies

Three hundred fifty ml. of I.B.'s plasma and 250 ml. of R.S.'s plasma, both with clinically diagnosed Felty's syndrome, were administered to two hematologically normal recipients. Within 15 minutes from the beginning of the infusion a significant fall of the total leukocyte count was observed in each recipient (Figure 2a). There was no selective depression of either granulocytes or mononuclear cells. In one case (plasma from I.B.), the transfusion was followed by shaking chills for 45 minutes, accompanied by an urge to urinate, and in both instances the recipients experienced a transient temperature elevation to 100° and 101° F., respectively (Figure 2a). Identical infusions of normal plasma administered two days following each experiment produced no systemic reaction or significant change in leukocyte count in either recipient (Figure 2b). The infusion of 250 ml. of plasma from A.T. (SLE) into a control recipient produced no significant change in the leukocyte count.

DISCUSSION

The study of leukocyte immunology in clinical investigations has been based chiefly on the *in vitro* demonstration of leukoagglutinins and *in vivo* transfusion or transplacental (41) transfer of antileukocyte factors. The problems and inaccuracies of the first method stem mainly from the difficulty in preparing adequate leukocyte

² We are grateful to Dr. T. E. Brittingham, St. Louis, Mo., for sending us this serum.

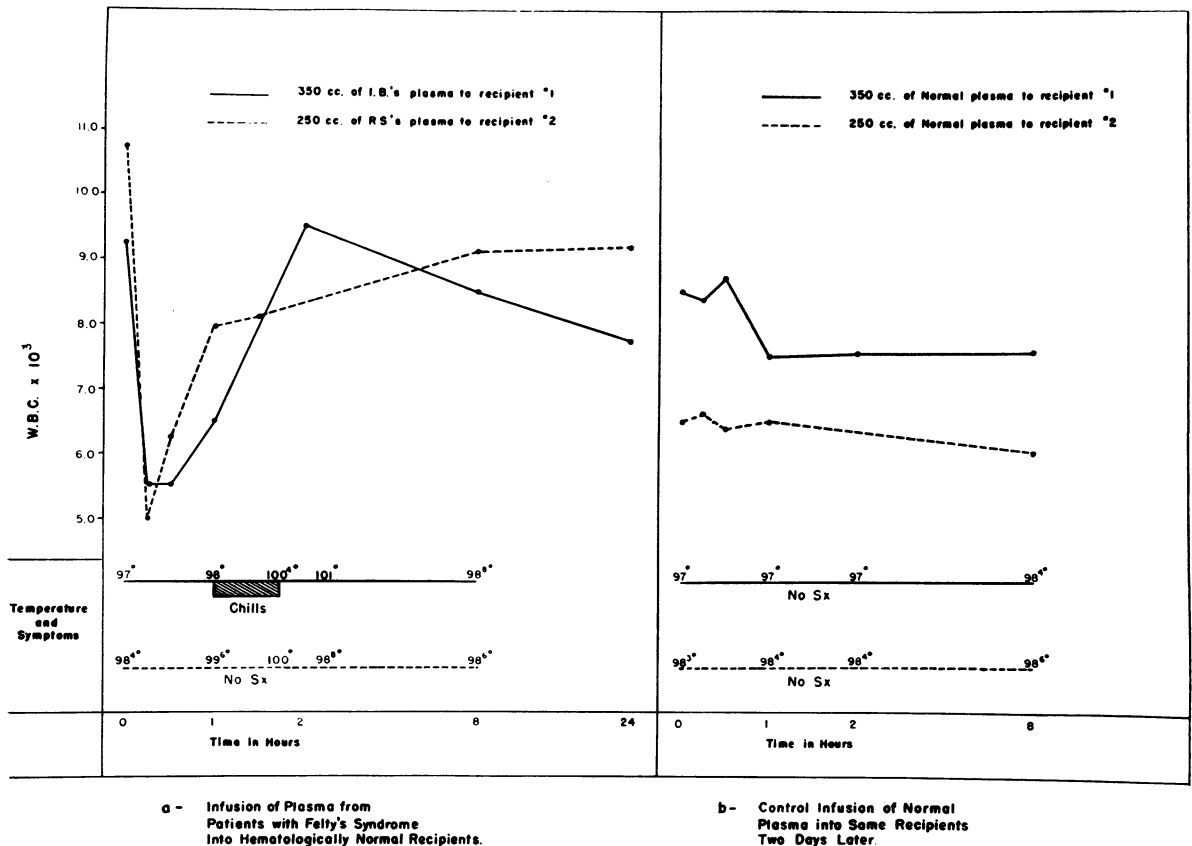


FIG. 2. RESULTS OF PLASMA INFUSIONS

In all cases the infusions were begun at zero time. They were terminated at 30 minutes in recipient No. 1 and at 60 minutes in recipient No. 2.

suspensions without causing injury and hence alteration of the cell surface, which results in false positive agglutination (6). In addition, agglutinations may occur from fibrinogen adsorbed on the surface of normal leukocytes (42), from leukergy (43), as well as from red and white cell clumps either secondary to erythrophagocytosis (28, 35) or nonspecific cellular adherence (44). The hazards (23) and inherent limitations of the *in vivo* studies preclude their extensive use in clinical practice. Neither technique is readily applicable to the study of autoimmune leukopenia or to the demonstration of incomplete and cell-bound leukocyte antibodies (25). Finally, it is difficult with either method to differentiate between immunologic factors directed against the various types of leukocytes, and both fail to detect specifically antinuclear and anticytoplasmic factors. The fluorescent antiglobulin technique is theoretically capable of circumventing these difficulties.

The use of this technique for the study of leukocyte immunology has been hampered by the fact that nonspecific fluorescent staining of the leukocyte cytoplasm occurs with labeled rabbit anti-human globulin (Coombs') serum (29, 33, 34, 45). The precise mechanism of this phenomenon is not clear, but it may be due to nonspecific adsorption of plasma proteins on the hydrophilic polar surface of the leukocytes (42). In our hands, nonspecific fluorescence occurring with untreated labeled antiglobulin made the differentiation of positive from negative preparations very difficult and often impossible. However, absorption of the labeled conjugate with acetone-dried rabbit bone marrow before use (33, 34) greatly diminishes or entirely eliminates the nonspecific cytoplasmic fluorescence (compare Figures 1b and 1d). This step greatly facilitates the detection of specific antinuclear reactions by providing definite contrast between nuclear fluorescence and cyto-

plasmic background (Figures 1a and 1c). Furthermore it may prove valuable in studying specific anticytoplasmic substances which could not be otherwise accurately quantified in the presence of this coexisting nonspecific fluorescence. Although an increased cytoplasmic localization was present when absorbed antiglobulin was superimposed on the sera of patients receiving multiple transfusions, it is evident that further carefully controlled studies are required to ascertain whether this observed difference is reproducible.

Previous animal experiments (46, 47) have indicated that two types of heteroimmune granulocyte antibodies may be produced. One of these is directed against the cytoplasm and may be obtained by injecting intact cells (47), whole cytoplasm (46, 48) or cytoplasmic fractions (46, 49). The antiserum thus produced appears to promote ingestion of the intact granulocyte with lytic effects upon the cytoplasm (46, 47) and is said to be specific for leukocytes (46). The other type of antibody may be produced by injecting nuclear material from granulocytes (48) or other tissues and hence appears to be relatively nonspecific (46). Although administration of this antiserum to animals produces a much less pronounced granulocytopenia than anticytoplasmic serum (48), when combined with normal granulocytes it is capable of producing "L.E.-like" cells (46). It has been recently reported by Friou, Finch and Detre (50) and Holman and Kunkel (51) that the fluorescent antiglobulin technique could be employed to demonstrate the interaction of the serum L.E. factor and cell nuclei and nucleoprotein. Moreover, it appears that nuclear material from many different species and varying human tissues can be made to participate in this reaction (45, 50-53). Our results have confirmed the work reported by others indicating the affinity of the serum of patients with SLE for leukocyte nuclei. Furthermore, in one instance, this test has proven more sensitive than the conventional L.E. cell preparation, anticipating the demonstration of the latter by at least two months. A similar case, with detection of "lupus globulin" preceding the L.E. cell test by two years, has recently been reported in detail by Friou (54).

Since 1924, when Felty (55) first described a syndrome consisting of leukopenia, splenomegaly and arthritis, there has been much speculation re-

garding the nature of this entity and the cause of the leukopenia. It is generally accepted that this represents a variant of rheumatoid arthritis (56-58). The leukopenia, often a neutropenia, has been variously attributed to chronic infection (57), hypersplenism (56, 58-60), leukocyte agglutinins (17) or unknown causes (61). It is well recognized that a positive L.E. cell test can be obtained in rheumatoid arthritis (54, 62-64), particularly in Felty's syndrome (65). In addition, the presence of small quantities of "lupus globulin" has been detected in patients with rheumatoid arthritis (54). The finding of positive nuclear fluorescence in 12 of our cases of rheumatoid arthritis is therefore not entirely unexpected. It is particularly noteworthy, however, that all 10 cases of Felty's syndrome, without exception, showed a strongly positive reaction indistinguishable, by our method, from that seen with SLE. To our knowledge this has not been previously reported. This finding offers further evidence of a basic relationship in these disease processes and encourages speculation that we may be dealing with a similar pathologic entity with a wide spectrum of manifestations. Thus, Felty's syndrome may be a connecting link which lies somewhere between uncomplicated rheumatoid arthritis and SLE.

Some authors have considered that the L.E. factor may be responsible for the development of the leukopenia commonly seen in SLE (46, 66). We would like to suggest that an antinuclear globulin may also be partly responsible for the leukopenia seen in Felty's syndrome. Further evidence that an antileukocyte factor in Felty's syndrome may be involved in the pathogenesis of the leukopenia was obtained from *in vivo* experiments. Definite, immediate, transient depressions of the leukocyte count accompanied by chills and fever, were obtained upon transfusion of 250 and 350 ml. of sera containing this factor. The quality and duration of this response are similar to those described by others (4, 6, 17, 67-69). Presumably the fever was secondary to the release of tissue pyrogens from destroyed leukocytes (6, 70, 71); however, notwithstanding sterile cultures, the possible presence of bacterial pyrogens cannot be entirely excluded (72).

Recent work has demonstrated that patients who have received multiple transfusions may de-

velop leukoagglutinins (23, 68, 73) or incomplete white cell antibodies (74) in their sera. Moreover, isoimmunization of a normal subject has been achieved by repeated injection of leukemic blood (23). The practical importance of this phenomenon in producing febrile transfusion reactions has been stressed, and their prevention by removal of leukocytes prior to transfusion is advocated (22, 68, 75). If an inference can be drawn from the previously discussed animal experiments (46-49), it would seem reasonable to find that these presumed human leukocyte isoantibodies are primarily directed against cytoplasmic antigens.

There are many other conditions in which anti-leukocyte substances have been demonstrated or suspected. The application of the fluorescent anti-globulin technique for the study of sera from such patients should be of help in elucidating the mechanisms of the leukopenia, the type of cells involved, the character of the substances responsible and their location on the cell.

SUMMARY

A method for studying leukocyte immunology by the fluorescent antiglobulin technique is described.

The results with sera from various disease states are reported. The data presented are consistent with the concept that human antileukocyte globulins may be directed against the nucleus or against the cytoplasm.

Antinuclear globulins were detected in the sera of all patients with systemic lupus erythematosus (SLE) and Felty's syndrome studied, and in two cases of apparently uncomplicated rheumatoid arthritis. These findings suggest that Felty's syndrome may be a connecting link of a disease spectrum involving simple rheumatoid arthritis and SLE.

Evidence that a circulating factor present in patients with Felty's syndrome may be involved in the pathogenesis of the leukopenia was obtained in two instances by plasma transfusions.

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