ANTIBODY PRODUCTION IN RHEUMATIC DISEASES. THE EFFECT OF BRUCELLA ANTIGEN *

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The possibility that a disordered immune response is related to the development of the rheumatic diseases has been an attractive hypothesis for investigation for years. Several investigators have attempted to demonstrate quantitative differences in the immune response between patients with these diseases and control subjects when they were inoculated with various antigens. Rantz, Creger and Choy (1) challenged patients with rheumatic fever and rheumatoid arthritis by injecting isologous red blood cells intravenously, and by the inoculation of influenza A and B vaccine. The patients with rheumatic fever responded with an increased titer of isohemagglutinins and in increased titer of antibodies to influenza virus compared with the control groups. There was no difference in the immune response to these two antigens between the rheumatoid arthritic patients and control subjects. In a prospective study, Rejholec (2) administered brucella vaccine subcutaneously to a group of 900 children. A small group developed very high titers of brucella antibody, measured by the indirect Coombs technique. All the children were followed clinically and a very high percentage of the hyper-reactors were shown subsequently to develop rheumatic fever; indeed, the only cases of rheumatic fever appeared in this hyper-reactor group. Miller, Kibrick, and Massel (3), by using influenza virus and typhoid O and H antigens, were unable to show hyper-reactivity in a group of patients with rheumatic fever. Other studies with pneumococcal polysaccharide by Quinn, Seastone and Dickie (4) and with diphtheria toxoid by Kuhns and McCarty (5) similarly showed no hyper-reactivity.

The present studies were initiated in the face of these conflicting data and with the following two objectives in mind: *1*) to determine whether differences in antibody response to an antigenic stimulus of a nonstreptococcal, presumably unfamiliar antigen could be demonstrated between control patients and patients with various rheumatic diseases; and *2*) to determine whether challenge by a specific heterologous antigen might influence one or another of the abnormal antibodies or antibody-like substances circulating in the blood of patients with rheumatic diseases.

MATERIALS AND METHODS

Nineteen patients with rheumatoid arthritis in all stages of activity, 11 patients with systemic lupus erythematosus, 5 patients with acute rheumatic fever, and 6 patients with acute glomerulonephritis formed the study group. Almost all patients with rheumatoid arthritis, rheumatic fever and systemic lupus erythrematosus were under chronic corticosteroid therapy. Twenty-seven patients with various nonrheumatic diseases (cerebral vascular accident, congestive heart failure, various neurological syndromes) served as hospitalized, chronically ill, control subjects. These control subjects were comparable in age, although predominantly male (Table I). The patients with rheumatic diseases, as one might expect in rheumatoid arthritis and systemic lupus erythematosus, were predominantly female. Forty apparently normal subjects, the kindred of nonrheumatic patients, acted as normal ambulatory control subjects.

Each patient was inoculated subcutaneously with 0.5 ml of brucella vaccine (Parke-Davis) containing *Brucella abortus* and *melitensis,* 2,000 million killed bacteria per ml. In most cases, blood samples were obtained before inoculation and at 1, 2 and 3 weeks after inoculation. In 2 patients, samples were obtained at 2-day intervals. In 12 cases, samples were taken once a week for periods of months. In many instances the following agglutinations were performed on the several samples of a single patient simultaneously.

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Antibrucella agglutinins were measured by the slide agglutination technique, with diagnostic febrile antigen for brucella (Lederle), according to the method of Huddleson and Abell (6). The sera of 2 control patients and 2 rheumatic patients were independently studied by Dr. D. Widelock of the Department of Health, New York City, and comparable titers of brucella antibodies were found. Isohemagglutinin titers were determined as described by Kabat (7) and antiglobulin tests were performed as described by Rosenfield, Vogel and Rosenthal (8). Lupus erythematosus cell preparations were made in accordance with the method of Lee (9), and antistreptolysin titers were done by the method of Rantz and Randall (10).

Antinuclear antibody titers were performed by a fluorescent antibody technique described previously (11) with this major modification: imprints of mouse spleen, liver and kidney were used as a source of nuclei, rather than chicken red cells. Slides so prepared were fixed in acetone for 10 minutes and used immediately with the usual indirect staining method. The fluorescent conjugates were absorbed twice for 1 hour with guinea pig liver powder in order to remove nonspecific fluorescence.

Anti-thyroglobulin antibodies were detected in inactivated sera by means of a slide agglutination method, utilizing thyroglobulin-coated latex particles prepared by Hyland Laboratories. The test was performed in undiluted serum and at a dilution of 1 : 10 in order to allow for any prozone phenomenon. Whenever the diluted or undiluted serum was positive, serial dilutions were done to determine the exact titer. Forty control sera from blood bank donors were negative and 19 of 20 patients with suspected thyroid disease were negative. One patient with clinical Hashimoto's disease had a positive serum. The positive control serum provided by Hyland Laboratories and considered very strongly positive had a titer of 1 : 64. Latex particle fixation was done by the method of Singer and Plotz (12). Influenza antibodies type A_2, B Lee, PR8, FM, and SW were measured in 17 selected sera of 5 patients with systemic lupus erythematosus by Dr. D. Widelock of the Department of Health of New York City.

Titers of all antibodies will be expressed as the reciprocal of the dilution. All of the antinuclear antibodies, lupus erythematosus preparations, and anti-thyroglobulin antibodies were done on all of the samples of each patient at the same time. Serum complement titrations were determined with slight modifications according to the technique described by Kabat and Mayer (13). Paper electrophoresis was performed in a Durrum-type cell (Manual of Paper Electrophoresis, model R, Beckman Instrument Co., 1958). The absolute values of different fractions were determined as the percentage of total protein measured by the biuret method or by refractometry.

Sedimentation characteristics of the antibrucella agglutinins, the rheumatoid factor, and the antinuclear antibodies were studied in a Spinco model L ultracentrifuge by 2 different procedures. Initially, serum diluted in saline was centrifuged at 40,000 rpm in a 40.2 rotor for 1 to 2 hours; after centrifugation, serial aliquots of 8 successive layers were removed consecutively from the top to the bottom of the tube. Afterward, better results were obtained by means of the zone centrifugation technique. This was done with the swinging bucket SW 39 rotor, using a sucrose density gradient, as described by Kunkel (14).

TABLE I

Control patients

Patient	Diagnosis	Age	Sex	Antibrucella titer Initial	1 wk	2 wks	3 wks
G.B.	As. ht. dis.	72	M	neg	320	640	80
A.B.	As. ht. dis.	64	M	neg	neg	neg	neg
S.T.	CVA*	78	M	neg	20	40	80
M.R.	Paget's	60	M	neg	neg	neg	
J.B.	Meningioma	50	M	neg	neg	neg	neg
E.C.	Bronchogenic ca.	64	M	neg	40	40	
B.	Metastatic brain ca.	67	M	neg	neg	20	40
De.C.	Bronchogenic ca.	50	M	neg	80	640	
D.G.	As. ht. dis.	58	M	neg	20	640	160
G.C.	Ca. of rectum	50	M	neg	160	640	160
M.L.	Pancreatitis	72	F	neg	neg	320	320
G.D.	Arteriosclerosis	69	M	neg	neg	neg	
J.M.	CVA	80	M	neg	40	160	80
J.M.	Arteriosclerosis	71	M	neg	neg	40	80
W.S.	Arteriosclerosis	64	M	neg	20	80	80
G.K.	CVA	70	M	neg	neg	80	20
J.S.	CVA	62	M	neg	40	80	160
J.T.	Hemangioma	39	M	neg	neg	80	160
A.M.	Syringomyelia	42	M	neg	160		320
V.J.	Congestive ht. dis.	40	F	neg	neg	160	
T.C.	Congestive ht. dis.	34	M	neg	80		
A.C.	Cirrhosis	60	F	neg	160		
F.H.	Rheumatic ht. dis.	44	F	neg	20	80	80
E.D.	Rheumatic ht. dis.	23	F	neg	80	80	40
T.H.	Pyelonephritis	32	F	neg	40		
M.H.	Psoriatic arthritis	39	F	neg	40		320
A.S.	Paralysis agitans	69	M	neg	40	80	40

* Cerebral vascular accident.

TABLE II

Antibrucella titers for all groups *

Group	Sample size	Geometric mean antibrucella titer Initial	2 wks	95% Confidence limits
Family members	40	0	210	150– 300
SLE	10	0	400	240– 660
RA	13	0	555	350–1,000
Other diseases	17	0	80	40– 155

* SLE = systemic lupus erythematosus; RA = rheumatoid arthritis.

RESULTS

A. Brucella antibodies

Table I summarizes the antibrucella titers in the hospitalized control subjects at 7, 14 and 21 days. The average geometrical mean titer was 80 at the end of 2 weeks. A summary table of all

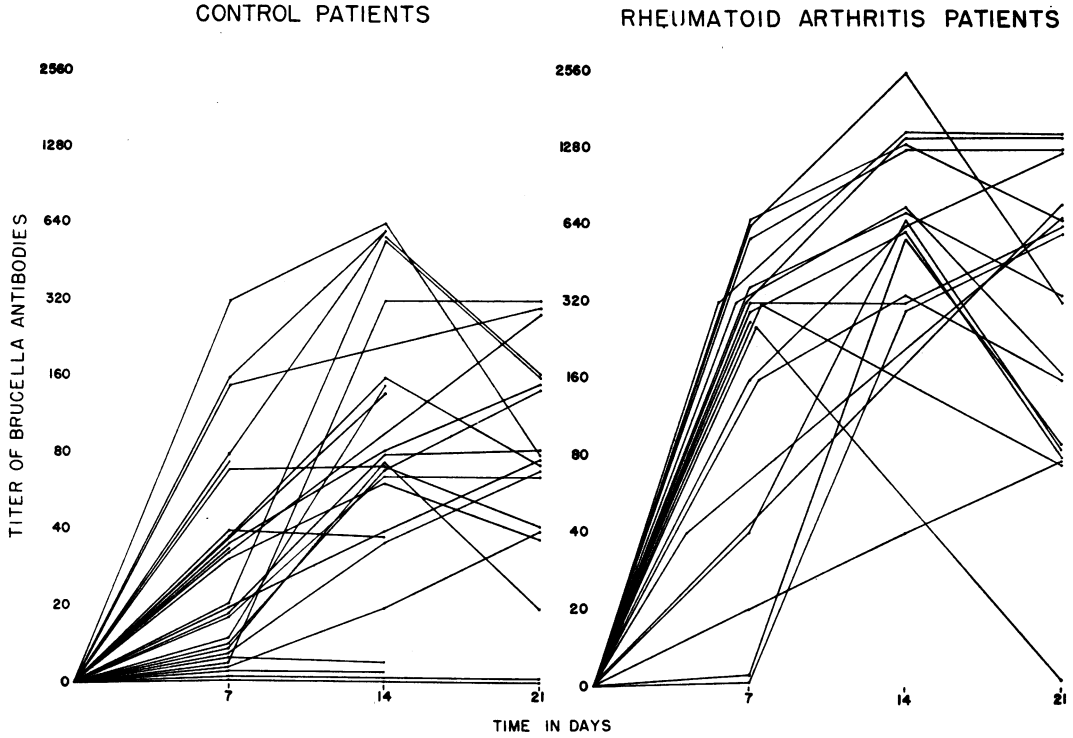

FIG. 1. ANTIBRUCELLA TITERS IN CONTROL SUBJECTS AND RHEUMATOID ARTHRITIS PATIENTS.

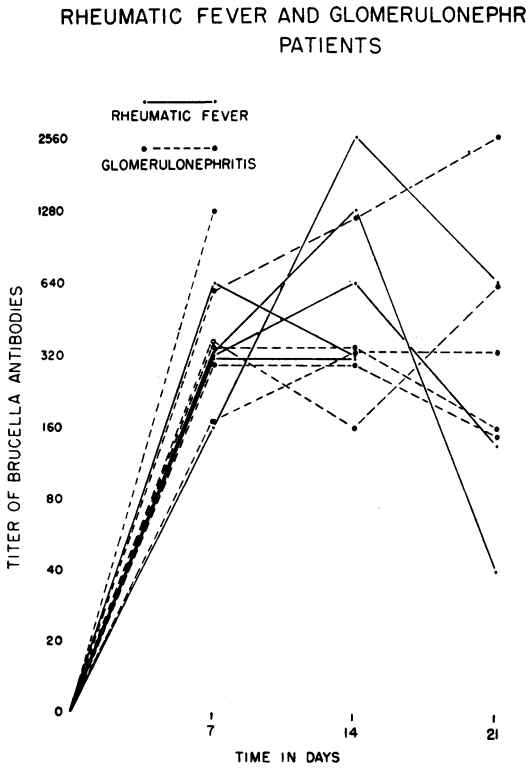

FIG. 2. ANTIBRUCELLA TITERS IN PATIENTS WITH RHEUMATIC FEVER AND GLOMERULONEPHRITIS.

groups at the 2-week interval shows that both the family control group and the hospitalized patient control group were significantly less reactive than the rheumatoid arthritis group[1] (Table II). Because the sample number in the systemic lupus erythematosus group was 10, the p value is not significant, but the difference between groups would be significant if the sample were larger.

Brucella antigen had been chosen for these experiments because it was presumed that it had not been met by the subjects and hence was satisfactory for stimulation. The possibility exists that for some of our patients the antigen was not unfamiliar. Of the total of 68 patients inoculated, three had positive antibrucella agglutinin titers before inoculation. These three, with titers of 20, 40, and 80 were in the systemic lupus erythematosus group. The mean titer at 2 weeks in the rheumatoid arthritis group was 555 and in the systemic lupus erythematosus group was 400. The small size of the rheumatic fever and glo-

[1] p Value for difference between systemic lupus erythematosus patients and normal family members = 0.075; p value for difference between rheumatoid arthritis patients and normal family members < 0.01.

TABLE III

Laboratory data on systemic lupus erythematosus patients

Patient	Antibrucella titers				LE cells	Antinuclear antibodies		Antiglobulin test		Isohemagglutinins		γ-Globulin		Latex particle fixation		Complement (50% units)		Anti-thyroglobulin antibodies	
	Initial	1 wk	2 wks	3 wks		Initial titer	2 wks	Initial	Later	Initial titer	2 wks	Initial	2 wks	Initial	2 wks	Initial	2 wks	Initial titer	2 wks
M.L.	20	20	1,280		neg	4	4	neg	pos. (2 wks)	B: 128	64	1.82	2.00	20	neg	61	48	neg	neg
J.H.	neg	640	1,280	1,280	neg*	16	32	neg	pos (4 wks)	A: 32 B: 64	128 128	1.31	1.05	neg	neg	76	60	neg	16
M.P.	neg	160	640	320	pos	32	16	neg	neg	B: 4	4	1.74	1.65	neg	neg			4	4
N.R.	neg	neg	80	neg	pos	256	128	neg	neg	A: 64 B: 64	32 16	2.52	2.49	neg	neg	100	70	4	32
R.W.	neg	320	640	320	pos	8	16	neg	pos (2 wks)	B: 64	32	1.00	0.94	neg	neg			neg	2
M.S.	40	160	320	1,280	pos	16	32	neg	neg	A: 128 B: 32	128 32	1.18	0.95	160	neg				neg
J.E.	neg	neg	160	40	pos	512	256	neg	neg	A: 64 B: 128	64 64	1.82	1.57	20	neg			neg	8
M.S.	80	80	320	320	pos	16	16	neg	pos.† (8 wks)	A: 16	8	1.18	1.34	neg	neg	52	55	neg	neg
M.M.	neg	320	320	320	pos	64	32	neg	neg	A: 64 B: 32	32 32	1.20	1.71	neg	neg	64	56		
J.R.	neg	320	1,280	640	pos	256		neg	neg			1.71		neg	neg				
T.A.	neg	20	320	320	pos	64	64	neg	neg	A: 32 B: 32	128 64	1.54	1.12	neg	neg			neg	neg

* Patient had positive preparations on previous examination.

† Positive Coombs occurred 4 weeks after polio immunization and 2 weeks after anamnestic brucella agglutinin rise from 160 to 640. Titers are expressed as the reciprocal of the dilution.

TABLE IV

Influenza antibodies *

Patient	Initial titer					Later†				
	A2	B Lee	PR8	FM	SW	A2	B Lee	PR8	FM	SW
M.P.	32	64	128	64	0	32	128	64	64	0
R.W.	32	0	0	32	0	32	0	0	16	0
M.M.	0	32	64	32	64	0	32	64	16	64
J.E.	0	0	0	64	0	0	0	0	512‡	0
M.S.	0	16	32	16	32	0	4	32	16	64

* Titers are expressed as the reciprocal of the dilution.
† These samples were taken at the time of maximal production of brucella agglutinins.
‡ This rise occurred 2 weeks after booster dose of brucella vaccine.

merulonephritis groups does not permit statistical evaluation, but these also showed high titers at 14 days (160 to 2,560; Figures 1 and 2). The three systemic lupus erythematosus patients who had initial positive titers showed in general no difference in response to brucella antigen inoculation from the other patients with this diagnosis. Comparison of the mean titers at Days 7, 14 and 21 showed that at the latter two days, patients in the rheumatic disease group had significantly higher titers than those in the control series, with values of less than 0.001 for rheumatoid arthritis. More frequent bleedings done on two patients showed that the peak response occurred between the eighth and tenth day after inoculation.

B. Effect of brucella antigen inoculation on production of other antibodies

Rheumatic fever and glomerulonephritis. No significant rise of antistreptolysin titer was noted in this group of patients.

Systemic lupus erythematosus (Table III). Isohemagglutinin titers, antinuclear antibodies, complement levels and total serum γ-globulin did not show appreciable change in these patients following brucella antigen stimulation. In 4 of the 11 patients in this group, however, transiently positive direct antiglobulin tests were observed during the peak immune response to brucella. In five patients with systemic lupus erythematosus, serial blood samples were examined for antibodies to various influenza viruses [2] (Table IV). Four of these patients showed patterns in their influenza antibodies which are not unusual for their age group in New York City; these patterns did not change during the immunization period. In one patient, however, there was an initial titer of 64 for the FM 1 strain. Two weeks after a subcutaneous injection of 0.1 ml of brucella antigen, this titer rose to 512, although titers to all of the other strains tested remained zero. It is possible that this patient had an infection with the FM 1 virus during this period, although this virus has not been isolated in New York City in recent years.

Anti-thyroglobulin antibodies were detected before immunization in two patients in titers of 4 and 64 (Table III). Two weeks after stimulation these antibodies were detected in three additional patients in titers of 2, 8 and 16, although the original two patients did not have any subsequent change in their titers.

Rheumatoid arthritis. Titers of isohemagglutinins and serum γ-globulin showed no significant change. One patient developed a positive Coombs test 8 weeks after brucella inoculation.

In seven patients of this group serial determinations of the latex particle fixation were made. In three of these seven there was significant parallel rise of latex particle fixation titer as the brucella agglutinin titer rose (Figure 3).

Since it is well known that the disulfide bridge of the macroglobulins can be disrupted by the addition of sulfhydryl-containing compounds with subsequent loss of antibody activity, mercaptoethanol was added to seven high-titer brucella and latex sera in 0.01 M concentration. There resulted a marked loss of agglutinating activity of both the brucella and latex system. Ultracentrifugation of these sera revealed that both the brucella agglutinins and the latex particle fixation activity resided in the 19S component and that the 7S component was inactive in both systems. Similarly, the brucella agglutinins resided in the 19S fraction of the control sera (Tables V and VI).

In order to differentiate clearly both 19S activities, the same seven test sera (with both a high titer of brucella antibodies and a high titer in the latex particle fixation) were repeatedly absorbed with brucella antigen until no brucella antibodies could be demonstrated. After absorption these sera still had their initial activity in the latex particle fixation system.

Before stimulation with brucella antigen no

[2] Courtesy of Dr. Daniel Widelock, Bureau of Laboratories, Department of Health, New York City.

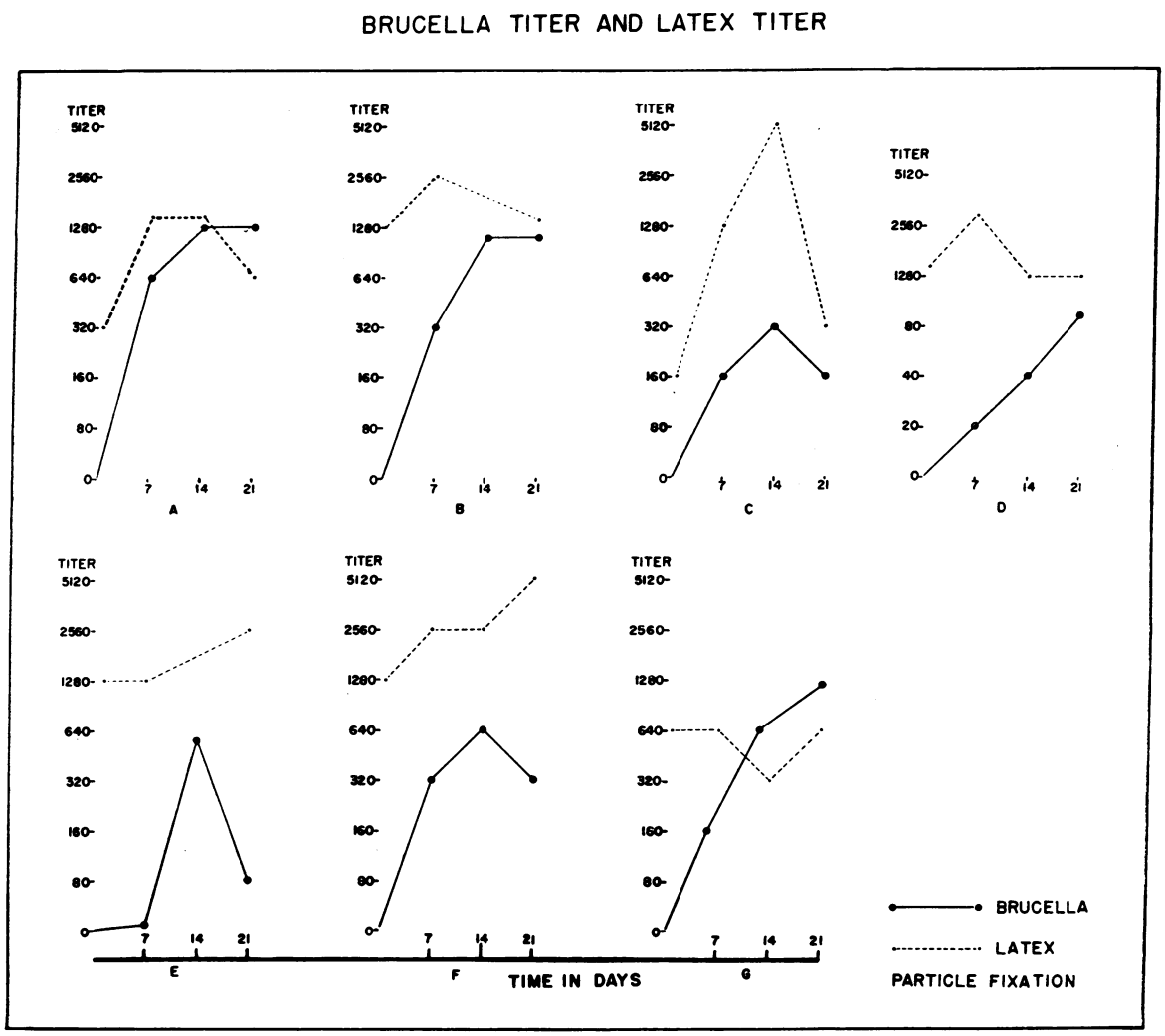

FIG. 3. BRUCELLA AND LATEX TITERS IN PATIENTS WITH RHEUMATOID ARTHRITIS.

patient with rheumatoid arthritis, of 15 tested, had detectable anti-thyroglobulin antibody. Approximately 2 weeks after inoculation these antibodies were detected in five patients in titers of 1, 1, 32, 2 and 2 (Table VII).

Control group. No latex particle fixation activity or lupus erythematosus cell factor was found in the initial serum of any of the control group. In the initial sera of 16 patients, no anti-thyroglobulin antibody was noted. In 13 of these patients no anti-thyroglobulin activity was found after inoculation with brucella antigen and during the period of brucella agglutinin response; γ-globulin in the control patients did not vary in the serial samples.

DISCUSSION

In this series of experiments, there were statistical differences in antibody response between the rheumatic disease patients and the control patients after brucella stimulation. The agglutination reaction used to detect the antibrucella agglutinins

TABLE V

Brucella antibody titer

Serum alone			Serum + mercaptoethanol (0.01)
R.W.	SLE	1:320	(−)
M.V.	RA	1:640	1:40
A.P.	RA	1:320	1:20
W.A.	RA	1:1,280	1:20

TABLE VI

Ultracentrifugation on sucrose gradient; latex and brucella titer of serum fractions

Serum alone			Serum + mercaptoethanol		
Layer	Latex	Brucella	Layer	Latex	Brucella
1	(−)	(−)	1	(−)	(−)
2	(−)	(−)	2	(−)	(−)
3	(−)	(−)	3	(−)	(−)
4	(−)	(−)	4	(−)	(−)
5	(−)	(−)	5	(−)	(−)
Pellet(+)	1:3,200	(+)1:1,600	Pellet(+)	1:200	(−)

A.P. (RA) Original serum: latex 1:2,560; brucella 1:640

was specific. Parallel agglutinations using *Salmonella typhosa* were uniformly negative. Antibrucella agglutinins in systemic lupus erythematosus patients were distinct from antinuclear antibodies, and in rheumatoid arthritis patients were demonstrated to be separate from rheumatoid factor. Further, rheumatoid factor had no enhancing effect when added to low-titer antibrucella sera.

The possibility that the agglutinating phenomenon was related nonspecifically to the presence of

TABLE VII

Antibody titers of rheumatic patients and patients with acute glomerular nephritis

			Antibrucella titers				Latex particle fixation titer		LE prep.	Anti-thyroglobulin titers	
Patient	Age	Sex	Init.	1 wk	2 wks	8 wks	Init.	2 wks		Init.	2 wks
Rheumatoid arthritis											
A.P.	19	F	neg	640	2,560	320	neg	neg	neg		
A.P.	35	F	neg	640	1,280	1,280	320	1,280	neg		neg
R.B.	65	F	neg	160	640	1,280	640	640	neg	neg	neg
E.G.	40	F	neg	320	320	640	neg	neg	neg	neg	neg
H.S.	40	M	neg	320	640		320	80	neg	neg	neg
I.R.	33	F	neg	320	640	80	neg	neg	neg		neg
M.G.	32	F	neg	640	1,280	640	40	20	neg	neg	1
W.A.	51	M	neg	320	1,280	1,280	1,280	2,560	neg	neg	neg
A.A.	48	F	neg	160	320	160	160	5,120	neg	neg	32
M.Q.	62	F	neg	40		640	2,560	2,560	neg	neg	
A.A.	30	M	neg	320		neg	neg	neg	neg	neg	2
L.N.	66	F	neg	320		1,280	neg	neg	neg	neg	2
P.S.	62	M	neg	20	40	80	1,280	2,560	neg	neg	neg
A.D.	45	F	neg	40	640	80	neg	neg	neg	neg	neg
R.C.	61	M	neg	320	640	320	1,280	2,560	neg		
P.W.	60	F	neg	320	640	160	neg	neg	neg	neg	neg
M.P.	60	F	neg	320		80	neg	neg	neg	neg	
D.R.	70	F	neg			320	neg	neg	neg	neg	
R.A.	64	F	neg	neg	640	80	1,280	2,560	neg	neg	1
Acute rheumatic fever											
F.W.	6	F	neg	640	1,280	320					
M.V.	17	M	neg	160	2,560	640					
P.B.	7	F	neg	320	640	40					
J.B.	11	M	neg	320	320						
G.R.	10	F	neg	320	1,280	40					
Acute glomerular nephritis											
S.H.	13	M	neg	40	320	160					
J.K.	9	F	neg	160	320	160					
T.M.	42	F	neg	640	1,280	2,560					
M.S.	12	M	neg	80	320	160					
M.W.	8	M	neg	1,280							
J.S.	22	M	neg	320	160	640					

complement was discarded, because in six instances simultaneous measurements of complement and antibrucella agglutinins revealed negligible complement activity in the presence of high agglutinating activity.

The present study confirms the suggestion that patients with rheumatic diseases may hyper-react to brucella antigen as previously reported by Rejholec (2). The data are in disagreement with the results obtained by other groups who have used diphtheria toxoid (5), pneumococcal polysaccharide (4) or typhoid vaccine (3). Resolution of this difference seems possible only on the basis that in the same individual there are different quantitative responses to various antigens. Brucella seems to be one antigen which is capable of eliciting a high antibody response in rheumatic disease patients. The known propensity for the brucella organism to produce tissue damage in mesenchymal structures, the target tissue in rheumatic diseases, possibly suggests a basis for understanding the present results.

What specific antigens in the brucella organisms are responsible for this hyper-response, and what other antigens share this property, are questions which are still unanswered. In the present series, patients with four distinct syndromes shared this capacity for developing antibody in high titer to brucella antigen.

Among the systemic lupus erythematosus patients, three had measurable antibrucella titers even before inoculation of the test brucella antigen. One of these patients was born in Malta, and may have had brucella infection. Its occurrence in the other two systemic lupus erythematosus patients suggests that perhaps the elevated baseline titer represents in them another abnormal response in antibody formation among the many which characterize this disease.

Of all the other antibodies studied, only four appeared to increase in titer secondarily to stimulation by brucella antigen. Four of 11 patients with systemic lupus erythematosus showed transiently positive direct *antiglobulin* (Coombs) tests; 1 of 5 systemic lupus erythematosus patients showed a marked rise in titer of an unusual *influenza antibody* (FM 1); and 3 of 7 rheumatoid arthritis patients showed possible (and 3 others showed suggestive) rises in *latex particle fixation titer,* admittedly a crude measure of rheumatoid factor. *Anti-thyroglobulin* antibodies occurred after brucella inoculation in 3 of 11 patients with systemic lupus erythematosus, and in 5 of 15 patients with rheumatoid arthritis. In all these instances the antibody activities measured were actually separate from the brucella agglutinins.

In all instances, when positive, the Coombs test became positive 2 to 8 weeks after stimulation; in two instances, the presence of an anti-red cell factor paralleled a sudden increase in titer of brucella agglutinins. The very high titer against influenza antibody occurred 2 weeks after a second dose of 0.1 ml of brucella vaccine.

Detectable anti-thyroglobulin antibody occurred in titers of 16, 2 and 8 in the systemic lupus erythematosus patients after inoculation. In two of the patients (J.H. and R.W.) anti-red cell antibody was transiently present at the same time. In the third patient (J.E.) the unusual titer to FM 1 strain of influenza virus, previously alluded to, had occurred. Two systemic lupus erythematosus patients had anti-thyroglobulin antibody before brucella inoculation; these showed no increase in their titer of anti-thyroglobulin antibody.

Prior to stimulation, no patient with rheumatoid arthritis had detectable anti-thyroglobulin antibody; 2 weeks later, 5 of 15 patients tested had detectable antibody. One of these 5 patients developed a titer of 32 at the same time that she developed an increase in the latex particle fixation titer from 160 to 5,120.

Thus, of a total of 11 antibody activities studied in rheumatic disease patients after brucella inoculation, significant alterations (appearance *de novo* or definite increase of titer) were observed in 4. These alterations occurred in 7 of 19 patients with rheumatoid arthritis and in 5 of 11 patients with systemic lupus erythematosus. Among the rheumatic disease subjects exhibiting this type of reactivity, there was a tendency for multiple changes in individual patients. Four patients with systemic lupus erythematosus and one with rheumatoid arthritis showed alterations in two different antibody activities (anti-red cell plus anti-thyroglobulin in three patients, anti-red cell plus anti-influenza in one patient, and rheumatoid factor plus anti-thyroglobulin in one).

The significance of this broad spectrum of antibody response to a single antigenic stimulus is

obscure. All rheumatic disease subjects did not show this phenomenon, but it seems likely that if more antibody activities had been measured, more reactive patients would have been found. It is possible, too, that the pattern which appears here—single antigenic stimulus yielding broad scattering of antibody response—may provide a basis for understanding the wide range of abnormal antibody activities found in systemic lupus erythematosus and rheumatoid arthritis. Autoimmunity has been thought to require some alteration of a normal tissue component to create antigenicity of this tissue (15) or the sudden availability of a normal tissue component for the immune system (16). The present results could be interpreted as indicating that the altered host response may be a basic defect in some "autoimmune" diseases.

There is some support for the hypothesis that this altered immune response may be genetically determined. This possibility has been suggested by the findings of serological and clinical abnormalities in the kindred of patients with systemic lupus erythematosus and rheumatoid arthritis (17–20).

In these studies no control patient showed response to brucella inoculation in any antibody activity other than that directed specifically against brucella; this lends support to the suggested hypothesis of an altered immune response. Hackett and Beech (21), however, have recently reported that during the course of active immunization with heterologous antigens in seven presumably healthy young women, two of these, with previously demonstrated anti-thyroglobulin antibodies, developed complement-fixing antibodies to liver and kidney tissue. Further studies of this sort with larger numbers of subjects may help to clarify this issue.

Seven of the "antibody" activities studied failed to participate in the "nonspecific" response. Attempts to analyze the two groups of antibodies defined by this response have not yet been carried very far. Antibodies have been classified in many ways: chiefly either according to the biological or physical nature of the antigen, or according to the physicochemical characteristics of the antibody. The 11 antibodies studied contained activities against isologous, heterologous and autologous antigens. The four "responding" antibodies do not segregate according to this classification; they include three autologous and one heterologous. Antigens may be classified as being particulate or soluble. This classification also does not serve to single out the "responding' group of antibodies.

Antibodies may be classified by their electrophoretic and sedimentation characteristics into three groups: γ-2 (7S), γ-1-M (or β-2-M) (19S), and β-2-A. Attempts are now being made to define the antibodies studied according to this classification. The antibrucella agglutinins in the present series have been shown to belong to the γ-1-M group, as does the rheumatoid factor. It seems possible that this approach may be a fruitful one and that all the "responding" antibodies may belong to this class. Studies are now under way to stimulate antibodies of another class in similar patients.

SUMMARY

1. Forty-one patients with various rheumatic diseases and 27 control patients were inoculated with brucella vaccine. As a group the patients exhibited a significantly greater rise in antibrucella agglutinins compared with the controls. Some overlap in both groups was present.

2. Alterations were noted in other antibody systems—anti-red cell (Coombs), anti-thyroglobulin, and possibly in the influenza antibody and rheumatoid factor—after this primary stimulation in some of the patients with rheumatic diseases, but no titers for these antibodies were noted in the control patients.

3. The effect of brucella antigen in these patients may be related to the damage that this organism can produce on mesenchymal tissue.

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