AUTOIMMUNE COMPLEMENT-FIXATION REACTION IN 1,014 PATIENTS

BY

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Gajdusek (1957, 1958), and Mackay and Gajdusek (1958) reported that fixation of complement occurred when sera from certain hospital patients were incubated with simple saline extracts of various human organs. They called this the "autoimmune complement-fixation reaction" (A.I.C.F.). It had no strict organ specificity, and it occurred most often in cases of liver disease, diffuse "collagen" disease, and macroglobulinaemia. Gajdusek (1958) gave his reasons for regarding the reaction as one between antibody and antigen, and for adopting the view that it is an "autoimmune" phenomenon.

We have looked for this A.I.C.F. reaction in the sera of 1,014 medical patients in the Royal Adelaide Hospital, and report the results, together with a discussion of some possible interpretations of the reaction other than on a basis of autoimmunity.

Materials and Methods

Source of Sera

Blood was taken as a routine from patients admitted to the beds of the professorial unit of the Royal Adelaide Hospital. Other sera were obtained from time to time from large groups of patients in other medical clinics of the hospital. A few specimens were taken selectively from cases of syphilis, yaws, cirrhosis, diffuse "collagen" disease, or from cases known to have abnormal serum protein electrophoretic patterns. Usually only one test was made on each patient, and this was soon after admission or in an acute phase of the disease, when diagnosis or management was under active consideration. Every one of the 1,014 cases was later reviewed and a diagnosis assigned without reference to the A.I.C.F. results, which were recorded separately.

Complement-fixation Test

Complement.—Pooled guinea-pig serum was stored in small amounts at -20°C .

Antigens.—Fresh organs, obtained surgically or at necropsy, were homogenized to a 10% suspension in physiological saline in a Waring blender, then centrifuged three times at 3,000 r.p.m. for 10 minutes at $+4^{\circ}\text{C}$, and the sediments discarded. The supernatant solution was the antigen, and was stored in small containers at -20°C for up to three months. Antigens were prepared from normal liver, kidney, lung, adrenal, and thyroid gland.

Diluent.—Calcium-magnesium saline was used throughout the test (Mayer *et al.*, 1946).

Technique.—A five-volume technique, using 0.2-ml. volumes in Wassermann tubes, was employed. Sera were inactivated for 30 minutes at 56°C before testing. Serum dilutions, antigen, and $2\frac{1}{2}$ HD₅₀ of complement, titrated previously in the presence of antigen, were allowed to stand overnight at $+4^{\circ}\text{C}$ and then for one hour at bench temperature. Two volumes of haemolytic serum were then added to each tube and incubated at 37°C for 30 minutes, after which the cells were sedimented by light centrifuging. 50% haemolysis was taken as the end-point. Each test was accompanied by a serum anticomplementary control, and known positive and negative sera were included in each batch of tests. Sera were initially screened at 1/10 and 1/80, and then titrated fully if complement-fixation occurred. A "positive result" was complement-fixation at 1/10 or a higher dilution. There were 10 sera among the 1,014 which were anticomplementary at 1/10 without antigen, and we regarded these as giving a negative A.I.C.F. reaction.

Results

The results are given in Table I, from which it is clear that many sera fixed complement with several antigens, rather than with one only; in fact, this was the rule and not the exception. Neither a characteristic pattern of activity with a group of antigens nor any reciprocal relationship between antigens could be seen on an ordinary inspection of the results, except that, of the five antigens used, kidney and liver were most regularly among the positive reactors. (Liver, kidney, adrenal, and thyroid antigens were used to test all the 1,014 sera, but only 903 of them were tested with lung. Consequently the appropriate reduced total of cases tested with lung is shown in each disease category in Table I.)

The allocation of patients to groups of diseases in Table I was made after a review of every case and without reference to the serological results. The association of diseases within the groups are apparent in most of them. Group 4 is one of the loosest, comprising the theme of vascular insufficiency in a broad way to include diabetes (with its arteriosclerotic complications) and haemorrhage, as well as cardiac and cerebral infarction, but not periarteritis nodosa, which is in group 7. Viral hepatitis is in group 8. Group 10 shows separately some conditions which may be of interest from the "autoimmune" point of view, but the bulk of this group is made up of undiagnosed cases, with some miscellaneous examples of comparatively rare conditions not classifiable under any of the other heads.

It is clear from an inspection of the table that the incidence of a positive A.I.C.F. reaction in the whole group of 1,014 hospital medical patients is much higher than in 164 apparently healthy blood donors, and that Gajdusek and Mackay's findings in liver diseases, "collagen" diseases, and paraproteinaemia are generally confirmed. Wishing to make useful comparisons between groups of people with different diseases (and not merely between disease and the healthy state), we decided that group 10 represented a suitable control collection of persons who were sick to a similar degree, being admissible to the same wards over the same period in the same hospital under the same physicians, as those in the other groups of more readily defined disease states. Consequently we have made a comparison

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between group 10 and each other group, using the χ^2 test, of the incidence of the A.I.C.F. reaction between the patients' sera and each antigen in turn. Those which vary significantly (for the numbers observed) from group 10 are shown in Table II.

The thyroid gland is the only organ which shows a good correlation between A.I.C.F. activity and clinical disease in the same organ as that from which the antigen derives. We are reporting on this elsewhere (Hackett *et al.*, 1960).

Some virus conditions other than viral hepatitis, particularly infectious mononucleosis, though not necessarily with a positive Paul-Bunnell test, seem to provoke A.I.C.F. activity. The positive reactions obtained in syphilis and yaws are not unexpected when it is considered how similar the A.I.C.F. technique is to the Wassermann reaction.

Group 4 and group 6 show respectively a possible deficiency of anti-kidney and anti-lung activity compared with group 10, but at a low ($P=0.05$) rate of significance.

An analysis by sex and age was made of 988 of the sera from patients where details of the age could be

TABLE II.—Probability (P) Values for the Statistically Significant Differences Between the Positive Results in Various Groups from Table I compared with Group 10

Group	Any One of the 5 Antigens	Liver	Kidney	Adrenal	Thyroid	Lung
1	<0.001	<0.001	<0.001	<0.001	<0.001	
3			0.05	<0.01	<0.05	
4			0.05			
6						<0.05
7	<0.01	<0.01	0.02	0.001	<0.001	<0.01
8	0.001	0.02	0.001	0.001		<0.001
9	<0.01		0.05		<0.002	

satisfactorily established. The results showed a higher incidence in females than in males in all decades below 60 years; above this the incidence was equal in the two sexes. The percentages are given in Table III. There was no clear tendency for the incidence of the A.I.C.F. reaction to rise or fall with increasing age in either sex.

We found that not one of the following blood-group antigens was exclusively present or absent in the red cells of all of 10 positive A.I.C.F. reactors taken at random: A₁, A₂, B, O; C, c, D, d, E; Le(a), Le(b);

TABLE I

Group	Disease	Total Tested with Liver, Adrenal, Kidney, Thyroid	Positive with any of these Antigens, or with Lung		Positive with Liver		Positive with Kidney		Positive with Adrenal		Positive with Thyroid		Total Tested with Lung	Positive with Lung	
			No.	%	No.	%	No.	%	No.	%	No.	%		No.	%
1	Syphilis and yaws (W.R.+)	15	13	87	9	60	8	53	12	80	11	73	4	2	50
2	Bacterial infections	68	7		4		5		4		1		65	3	
	Tuberculosis	11	2		2		0		0		1		11	1	
	Total	79	9	11	6	8	5	6	4	5	2	3	76	4	5
3	Viral infections	53	13	25	11	21	13	25	9	17	11	21	53	7	13
4	Diabetes	21	3		3		2		2		2		21	2	
	Peripheral vascular disease, Raynaudism	8	1		0		0		1		0		8	0	
	Atherosclerosis, coronary artery dis., infarcts of heart and brain, cerebral haemorrhage	80	9		5		5		5		5		79	4	
	Peptic ulcer, gastro-intestinal haemorrhage	33	5		3		2		3		4		30	2	
	Total	142	19	13	11	8	10	7	11	8	12	8	138	9	7
5	Carcinoma, sarcoma	176	28	16	14	8	19	11	14	8	10	6	133	12	9
6	Reticuloses	37	9		4		5		4		1		22	0	
	Lymph. leukaemia	10	2		1		2		2		0		10	0	
	Myel.	10	2		1		2		1		0		10	0	
	Myeloproliferative disorders	15	0		0		0		0		0		15	0	
	Para- and dys-proteinaemia	12	4		2		4		2		3		12	2	
	Total	84	17	20	8	10	13	15	9	11	4	5	69	2	3
7	Dissem. L. E.	13	10		8		8		7		9		12	7	
	Idiopathic acquired haemolytic anaemia	6	3		3		2		2		2		5	3	
	Rheumatoid arthritis	31	10		4		7		5		1		24	3	
	Rheumatic fever	18	1		0		1		0		1		18	0	
	Other diffuse "collagen" diseases	15	4		4		3		3		9		13	3	
	Total	83	28	34	19	23	21	25	17	20	22	27	72	16	22
8	Cirrhosis	60	21		14		19		13		8		56	14	
	Acute hepatocellular disease	18	6		3		5		4		4		16	6	
	Total	78	27	35	17	22	24	31	17	22	12	15	72	20	28
9	Thyroid diseases	69	24	30	11	14	16	20	8	10	16	20	73	10	14
10	Glomerulonephritis	15	3		3		3		2		2		13	1	
	Asthma	19	1		1		1		1		1		17	5	
	Hypersensitivity, allergy	5	0		0		0		0		0		5	0	
	Deficiency anaemias	19	3		0		1		1		1		19	0	
	Addison's disease	2	0		0		0		0		0		2	0	
	Nephrotic syndrome	6	1		0		1		1		1		6	0	
	Miscellaneous and undiagnosed	169	31		21		21		9		16		153	18	
	Total	235	39	17	25	11	30	13	14	6	21	9	213	20	9
	Grand total	1,014	217	21	131	13	159	16	115	11	121	12	903	102	11
	Healthy blood donors	164	13	8	8	5	9	6	8	5	3	2	164	6	4

TABLE III.—Age Incidence of the A.I.C.F. Reaction Shown as the Percentage of Positive Results in 455 Male and 533 Female Patients

Percentage Positive	Age Groups (Years)					
	0-40		41-60		61-100	
	M	F	M	F	M	F
With any of the five antigens . .	15	22	13	24	21	21
With liver	9	13	9	13	12	13
" kidney	11	17	9	17	17	16
" adrenal	11	13	8	10	9	11
" thyroid	7	14	7	10	8	10
" lung	6	15	5	14	14	11
Total No. in each group . .	138	149	172	189	145	195

M, N; Fy(a); Jk(a). All 10 were e-positive and Kell-negative, but this would be so with over 90% of the patients in the wards.

We found, as did Gajdusek, that the antigenic activity of the extracts was destroyed by heating to 60° C. for 30 minutes. Unlike him, we found that the activity was not removed by centrifuging at 65,000 *g* for 120 minutes, and so we have concluded that at least some of the antigenic activity in our preparations resides in soluble components smaller than cell microsomes.

Discussion

These findings have confirmed the clinical correlations found by Gajdusek and Mackay. We believe they were right to interpret this "A.I.C.F. reaction" on an "autoimmune" basis. The prevailing climate of opinion suggests that autoimmunity in some auto-aggressive form may be the underlying mechanism in many diseases, particularly some in which this reaction is frequently observed. At the same time there are other possible interpretations which we now consider.

Lipid-lipid Interactions, and Anti-lipid "Biological" Reagins

The lipid levels found in plasma can vary physiologically from one individual to another, between the two sexes, and pathologically in conditions such as hepatocellular disease, nephrosis, diabetes, and atheroma. This applies to characterized lipids such as cholesterol, cephalin, and lecithin, but the same may be true, and for other pathological conditions, of various fats, fatty acids, steroids, bile acids and salts, phospholipids, glycolipids, and lipoproteins.

All cells contain lipids, and the simple "antigen" extracts made for the A.I.C.F. reaction are rich in derivatives from broken cells. If certain tissues have peculiar combinations of lipids these would probably be characteristically represented in the extracts.

The Wassermann reaction (and similar complement-fixation tests for syphilis) is a refined prototype of the A.I.C.F. reaction in the sense that an original simple liver extract has now been replaced by purified cardiolipin. Such reactions are very sensitive to the addition of other lipids, so that a mixture of lipids can show some special activities *in vitro* which its individual components will not. Rapport *et al.* (1959) use the phrase "lipid-lipid interactions" to describe this.

The "biological" false-positive complement-fixations encountered in non-treponemal disease can now be identified by showing that the sera lack antitreponemal activity in the immobilization test. But Kent *et al.* (1958) have shown that such false-positive reactions can

be distinguished from true syphilitic ones in ordinary complement-fixation or flocculation tests with cardiolipin antigen, by adding specific amounts of lecithin which at different concentrations lead to a selective reactivity of either the syphilitic or the non-syphilitic anti-lipid. Such an observation makes a valuable practical distinction but does not elucidate the nature of the "biological" anti-lipid reagent. Nevertheless, it suggests how the A.I.C.F. reaction, with its lipid or lipid-rich antigens and probable sensitivity to other lipids in the serum, might depend on a reagent of the same type, and show non-immunological variation in complement-fixation among sera from patients with plasma-lipid abnormalities.

Eastwood (1920), in a review of early experimental work on the nature of the Wassermann reaction, showed how much of this was based on the opinion (which it appeared to support) that the syphilitic reagin was not an antibody arising *de novo*, but a constituent of normal plasma which was physically altered in syphilis and thereby changed from a previously masked state. Mackie and Watson (1926) and Mackie and Finkelstein (1928) showed that the Wassermann reagin was a serum constituent which could be found in heat-sensitive form, being destroyed at 55° C. in many, if not all, normal human sera, but was characteristically heat-stable at 55° C. in the sera of rabbits and certain other animals. In human syphilis it seemed to acquire a heat-stable form so that it survived the "inactivation" or heat treatment (56° C. for 30 minutes) that always preceded the serological tests. Kahn (1950) claimed that a heat-stable "syphilitic" antibody could be found in nearly all human sera if a sufficiently delicate technique was used. If the foregoing is true, the role of this specific globulin reagent (heat-sensitive or otherwise) in the normal body must be considered. If it is present in all people, and is only multiplied or altered physically by disease in some way which does not change its immunological specificity, then it cannot be looked on as an autoaggressive agent; it must have a physiological function, even if the term "autoantibody" is used to describe it.

Kidd and Friedewald (1942) have shown that heat-stable complement-fixing antibodies reacting with simple saline extracts of various organs, particularly liver and kidney, can be found in most rabbit sera, and we have confirmed this. Most rabbits, even though bred in captivity, are not diseased according to current concepts of pathology, so here is an indication that the presence of A.I.C.F.-type antibodies is a normal feature in some vertebrates. If the same were true of man, variations in heat stability combined with lipid sensitivity as outlined above could produce many of the patterns shown by the A.I.C.F. reaction in health and disease without invoking acquired autoimmunity. We are at present planning to investigate the effects of adding lecithin or cholesterol to the A.I.C.F. reaction, and of using complement-free unheated sera.

Enzyme Activity

Extracts used as antigens in the A.I.C.F. reactions must contain cell enzymes. Beeson and Rowley (1959) have shown that kidney has anticomplementary activity, probably because renal glutaminase liberates ammonia which destroys the fourth component of complement. If the sera of certain sick people contained relevant coenzymes or activators, it is conceivable that an apparently specific removal of complement might occur

when serum and "antigen" were mixed. This is another possible non-immunological explanation of the A.I.C.F. reaction. It could be investigated by the use of enzyme inactivators.

Aggregated Globulins

The lists of diseases in which the A.I.C.F. reaction is most commonly positive are similar to those in which "biological" false-positive complement-fixation tests for syphilis are most frequently found (Moore and Mohr, 1952), and are also similar to those where positive results in non-rheumatic conditions are obtained with ultra-sensitive elaborations of the Rose-Waaler test such as the latex-fixation reaction (Dresner and Trombly, 1959). There are indications that such reactions all detect a similar feature in serum which is the presence of abnormal large-molecule plasma globulins, perhaps produced by simple aggregation of smaller normal globulins. The tendency to produce these macroglobulins may be inherited, and be more common in women than in men; it may be accentuated by chronic hepatocellular disease, and may predispose to arthritis and the development of other symptoms associated with diffuse "collagen" disorders. Moore and Lutz (1955) produced evidence showing that in women a "biological" false-positive test for syphilis was often followed within a few years by the development of disseminated lupus erythematosus. This is in accord with the view of Haserick (1955), whose clinical studies led him to conclude that there was a "diathesis" or predisposition to this disease.

Autoaggressive or Not?

If the A.I.C.F. reaction is due to the activity of a macroglobulin or to that of a normal or "natural" reagin (which in the light of this discussion may amount to the same thing), the question remains whether or not it is an antibody-antigen reaction. It may not be, if all true antibodies arise only after classical "non-self" antigenic stimulation. However, if patterned globulins can arise without specific antigenic stimulation in some such way as Burnet (1959) visualizes, through the function of a special or uncontrolled clone of cells, then the reaction can be looked on as the equivalent of one between antibody and antigen in a broad sense, and must be regarded as playing a possible autoaggressive part in the aetiology of the diseases with which it is associated. However, an autoaggressive role for such globulins is not the only possible one, and on present evidence is not substantiated. "Autoantibodies" can sometimes be found in apparently healthy people. There may even be physiological patterned antibody-like globulins which play a normal part in the body's economy, and if this is not kept in mind their activity *in vitro*, in reactions like the A.I.C.F., may wrongly be taken as good evidence for the existence of an aggressive autoimmune process.

Summary

The sera of 1,014 mixed hospital medical patients have been tested for the "autoimmune complement-fixation" (A.I.C.F.) reaction described by Gajdusek (1957, 1958), using simple saline extracts of normal liver, kidney, lung, adrenal, and thyroid gland as antigens.

Some positive reactions were found in most common diseases, but the clinical correlations noted by Mackay and Gajdusek (1958) have been confirmed in that positive reactions, usually with more than one antigen, were found with significantly greater frequency in the

sera of cases of liver disease, "collagen" disease, and paraproteinaemia; and also in syphilis and yaws, some viral conditions, and thyroid disease. The thyroid was the only organ which showed some correlation between regular complement-fixation with a particular organ extract and clinical disease in the same organ.

Autoimmune interpretations of the A.I.C.F. reaction are valuable, but others which are discussed above should be kept in mind. These are the possibilities that the effect is a lipid-lipid interaction, or an enzymic artifact, or a phenomenon produced by the practice of heating sera to inactivate them before complement-fixation tests are performed, or that it represents a non-immunological alteration in specific globulins.

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Summing-up a symposium on the prevention and the control of heart disease held last autumn in Atlantic City, N.J., Dr. IRVING S. WRIGHT said: "There are now approximately two million people in the United States who have had strokes as far as we can tell from comparable studies. . . . For the sake of this discussion, we may say that in 80% of the two million a clot has been the critical factor. The remainder have occurred on the basis of haemorrhage, aneurysms, and various other pathological conditions. A million people or more per year suffer from myocardial infarctions. We do not know the exact figure because, as I have said, many are unrecognized. In 73% of the cases autopsied in our study of 1,031 cases of infarction the final closure was due to a clot (a thrombosis). This was the terminal event. We do have weapons to deal with this situation. Studies on anticoagulant drugs have shown in many countries of the world . . . that the usual untreated death rate can be reduced by a third and the complication rate during the first six weeks by approximately four-fifths." (*Amer. J. publ. Hlth*, 1960, **50**, No. 3.)