Diversity of Human Antibodies to TRIC Agents (Chlamydiae) Detected by Different Serologic Procedures* (33725)

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Group-reactive and strain-specific antigens have been domonstrated in some chlamydiae. Rake *et al.* (1) established that trachoma-inclusion conjunctivitis (TRIC) agents shared a common heat-stable group-reactive antigen with psittacosis, lymphogranuloma venereum (LGV) and other members of the group. The group-reactive TRIC antigen fixed complement in the presence of sera obtained from patients infected with LGV or trachoma agents (2). The complement fixation (C'F) test employing boiled suspensions of egg-grown chlamydiae as group antigen has been employed widely in the diagnosis of chlamydial infections. Group antigen in infected cells was detected also by immunofluorescence (3). On the other hand, when fluorocarbon-purified particles of chlamydiae were used as antigen in indirect fluorescent antibody (IFA) tests, the reaction exhibited some strain-specificity, and permitted the separation of a psittacosis strain from an LGV strain, or of certain TRIC strains from others (4). The absorption of human or animal antisera to TRIC agents with selected chlamydial antigens permitted some antigenic grouping of strains and suggested, again, the existence of strain-specific antigens (5, 6). These reports suggest that immunofluorescence may detect either group-reactive or strain-specific antibodies, or both, depending largely on the nature of the antigen.

The present investigation was undertaken in an attempt to determine whether the C'F and IFA methods measure the same or different populations of antibodies following TRIC infection and to determine the relative strain-specificity of these antibodies. For this purpose, cross-absorption experiments were performed with human antisera to 3 strains of TRIC agent using Bio-Gel and fluorocarbon-purified antigens. The findings suggest that the C'F and IFA methods measure 2 distinct populations of antibodies, and that the C'F antibodies carry the group reactivity whereas the IFA-reactive antibodies are strain-specific.

Materials and Methods. Antisera. The inoculation and observation of volunteers and the preparation of antisera have been described by Jawetz *et al.* (7) (see Table I).

Antigens. Complement fixation (C'F) antigen. This was a group-reactive antigen prepared by treating trypsinized suspensions of TRIC-infected yolk sac material with 1% sodium deoxycholate solution followed by repeated differential centrifugations according to the method of Ross and Jenkin (9).

Fluorocarbon-purified antigens. Slide antigens for immunofluorescence studies were prepared by treatment of infected yolk sac material with fluorocarbon according to the method of Hanna and Bernkopf (4) and Bio-Gel-purified particles as described by Isa (10). Particle counts were performed according to the method of Reeve *et al.* (11). Homogeneity of the particle suspension was judged by the Giemsa and Gimenez staining procedures. The IFA tests were performed according to the method of Hanna and Bernkopf (4), and C'F tests as described by Isa *et al.* (12).

Absorption of human serum with TRIC agent antigens. Selected sera from volunteers who had been experimentally infected with strains IC Cal 3 or IC Cal 8 and sera from a patient naturally infected with strain ASGH

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TABLE I. Classification of Agents.

TRIC strain	Official designation (8)	Isolated by
ASGH	TRIC/ /USA-Cal/Cal-2/OT	Hanna *et al.*, Calif., 1959
IC Cal 3	TRIC/ /USA-Cal/Cal-9/ON	Hanna *et al.*, Calif., 1960
IC Cal 8	TRIC/ /USA-Cal/Cal-15/ON	Hanna *et al.*, Calif., 1961

were inactivated at 56° for 30 min and were then absorbed with antigen preparations from the same agents. Absorbing antigens from IC Cal 3, IC Cal 8, or ASGH strains were prepared by either fluorocarbon purification or gel filtration of particles through Bio-Gel A-150. Absorptions were performed as follows:

(a) Approximately 3.5×10^{10} particles were centrifuged at 12,500*g* for 10 min. The supernatant fluids were discarded.

(b) To the pellet 0.5 ml of human serum was added and the particles were resuspended in the serum.

(c) The serum-antigen mixture was incubated at 37° for 1 hr and at 4° overnight.

(d) Particles were removed from the absorbed serum by centrifugation at 50,000*g* for 15 min. Serum antibody activity was tested by the C'F and IFA methods.

Immunofluorescence slides were read by two independent observers who were unaware of the nature of the serum or the absorbing antigen. The end point was taken as the highest serum dilution which both observers recorded as positive.

Results. Antibody Response. The C'F and IFA tests for detection of antichlamydial an-

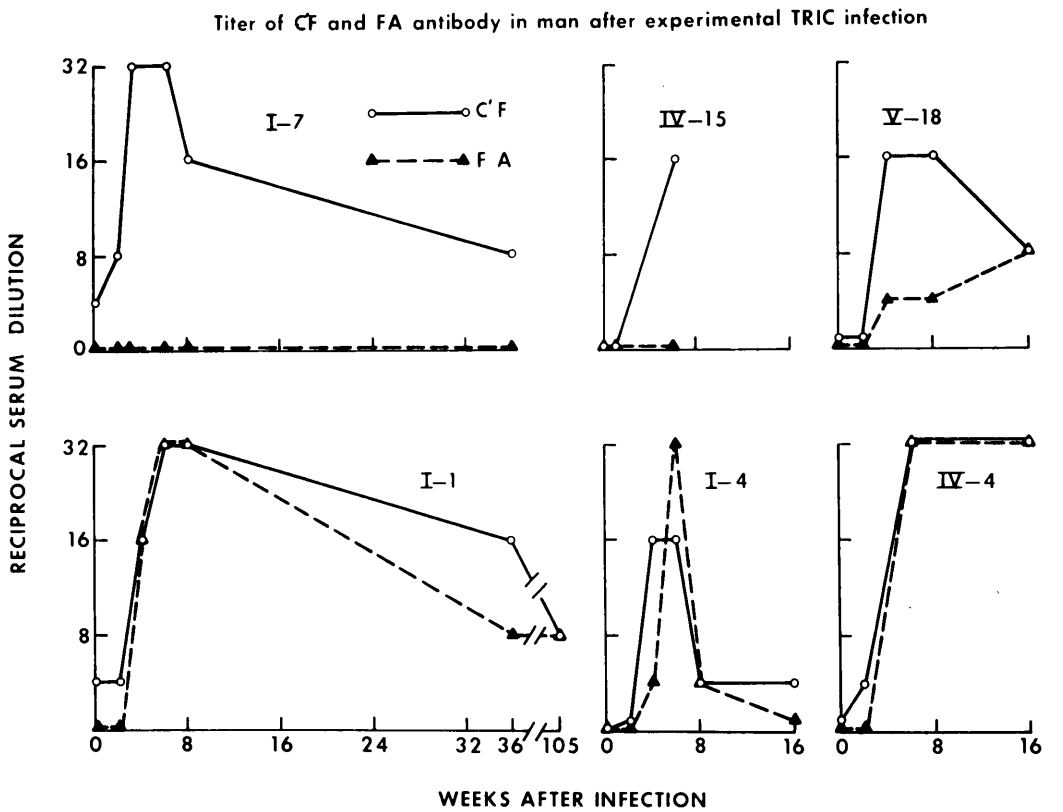

FIG. 1. Development of TRIC antibodies as determined by C'F and IFA in 6 experimentally infected human volunteers.

TABLE II. Titers of Apparent "Strain-Specific" TRIC Antibodies in Unabsorbed Human Serum.

Volunteer	Infectious agent	Weeks after infection	C'F[a]	Antibody titers IFA antigens		
				IC Cal 3	IC Cal 8	ASGH
I-9	IC Cal 3	5	64	64	64	16
IV-4	IC Cal 3	5	32	32	8	0[b]
IV-6	IC Cal 3	6	16	32	0	ND[d]
X-6	IC Cal 8	4	32	8	32	8
X-9	IC Cal 8	4	16	16	64	16
VI-22	IC Cal 8	4	64	128	128	128
VI-20	IC Cal 8	8	32	128	128	32
Patient ASGH[c]	ASGH	?	8	4	4	32
		?	8	4	4	64
		?	4	4	4	64

[a] C'F antibody titers measured vs group antigen prepared from strain ASGH.

[b] Serum negative when tested undiluted.

[c] This patient presumably infected naturally with the ASGH strain 20 years prior to isolation of the agent.

[d] ND = not done.

tibodies were applied to sera obtained from volunteers at the time of their inoculation and at intervals thereafter. Figure 1 shows the data from 6 volunteers whose sera were studied for up to 105 weeks following experimental inoculation. Although peak antibody titers were reached 4–6 weeks post infection in most cases by both methods, some variation in detection of antibodies was apparent between the 2 tests. Most of the volunteers produced antibodies demonstrable by both C'F and IFA methods. Several persons failed to produce antibodies detectable by either method for up to 4 weeks postinfection. Two volunteers infected with IC Cal 3 produced antibodies demonstrable by C'F but not detectable by the IFA method during the observation periods of 6 and 36 weeks (Fig. 1 volunteers I-7 and IV-15).

All C'F tests were performed with antigens prepared from 1 trachoma (ASGH) strain. Some degree of strain-specificity was apparent when individual antisera were tested against homologous and heterologous slide antigens by IFA.

Table II depicts the results of comparative studies between C'F and IFA of antisera to IC Cal 3, IC Cal 8, and ASGH when tested against the homologous and heterologous antigens. Although comparable C'F antibody titers existed in certain sera of some volunteers, IFA titers varied and higher titers were often exhibited with the homologous slide antigen (volunteers IV-4 and X-6, IV-6 and X-9, Table II). A more pronounced strain specificity was observed in sera from patient ASGH. By IFA his sera exhibited only minimal cross-reactivity with heterologous antigens (Table II). These IFA experiments suggested the presence of at least 2 populations of antibodies in the serum of individuals infected with TRIC agents. One population of these antibodies appeared to carry group-reactivity and was detectable by C'F, whereas the other appeared to be at least in part strain-specific and was detectable by IFA.

Cross-absorption. If C'F antibodies react with the group-reactive antigen(s) and are indeed distinct from those detectable by IFA, absorption of serum with heterologous chlamydial antigens should result in a greater reduction of the C'F antibody titers than of the IFA titers. Table III illustrates the results of cross-absorption studies in which

TABLE III. Titers of Apparent "Strain-Specific" TRIC Antibodies in Absorbed Human Serum.

			Antibody titers[a,c]							
			Unabsorbed		Absorbing antigens[f]					
					IC Cal 3		IC Cal 8		ASGH	
Volunteer	Infectious agent	Weeks after infection	C'F	IFA	C'F	IFA	C'F	IFA	C'F	IFA
I-9	IC Cal 3	5	64	64	0[b]	0	4	4	1[d]	16
IV-4	IC Cal 3	5	32	32	4	0	4	4	4	8
		16	32	32	0	0	4	8	4	8
X-6	IC Cal 8	4	32	32	4	8	4	0	1	16
X-9	IC Cal 8	4	16	64	0	4	0	0	0	16
VI-22	IC Cal 8	4	64	128	4	1	4	1	4	4
		8	32	128	4	0	4	0	1	16
		12	32	64	4	1	4	0	1	16
VI-20	IC Cal 8	8	32	128	4	1	4	0	4	4
Patient ASGH[e]	ASGH	?	8	32	0	16	0	16	0	0
		?	8	64	0	64	0	64	0	0
		?	4	64	0	64	0	64	0	0

[a] C'F antibody titers measured vs group antigen prepared from strain ASGH.

[b] Serum negative when tested undiluted.

[c] IFA titers of unabsorbed serum measured vs homologous antigen used for experimental infection.

[d] Serum positive when tested undiluted.

[e] This patient presumably infected naturally with the ASGH strain 20 years prior to isolation of the agent.

[f] Absorbing antigen dose equivalent to 3.5×10^{10} particles purified by fluorocarbon/0.5 ml serum.

high-titered human anti-TRIC antisera were absorbed with antigens prepared by fluorocarbon purification from the 3 strains. Initial studies showed that absorption with Bio-Gel-purified antigens reduced the C'F antibody titers of homologous and heterologous antisera approximately 16-fold. Absorption with the homologous Bio-Gel-purified antigens reduced the IFA titers more than heterologous antigens. When the serum of volunteer X-9 (infected with IC Cal 8) was absorbed with Bio-Gel-purified antigens of ASGH, IC Cal 3 or IC Cal 8, the C'F antibody titers were reduced to zero (16-fold) while the IFA titers were reduced 2-, 4-, and 8-fold, respectively. Absorption of the same serum with fluorocarbon-purified antigens of the homologous strain (IC Cal 8) reduced both the C'F and IFA antibody titers to zero; with absorbing antigens prepared from ASGH or IC Cal 3, the C'F antibody titers were abolished and the IFA titers were reduced 4- and 16-fold, respectively. Comparable results were obtained when the serum of volunteer I-9 (infected with IC Cal 3) were obtained upon absorption with Bio-Gel- or fluorocarbon-purified antigens. Absorption with fluorocarbon-purified antigens permitted better separation of inclusion conjunctivitis strains and the ASGH (trachoma) strain than with Bio-Gel-purified antigens. Therefore absorptions with the latter antigen perparations were discontinued. Sera from the patient infected with the ASGH strain were absorbed with fluorocarbon-purified antigenic preparations obtained from all 3 strains. Table III shows that absorption with antigens of the homologous or heterologous strain abolished the low C'F antibody titers of each of these sera. However, in contrast to previous

findings with sera from volunteers infected with IC Cal 3 or IC Cal 8, absorption with heterologous strain antigens failed to remove significant amounts of the IFA-reactive antibodies. Absorptions with the homologous (ASGH) strain completely abolished the IFA-reactivity of these sera. The above experiments clearly indicate that the strain-specific antibodies demonstrable by IFA are indeed distinct from the group-specific antibodies demonstrable by C'F.

Discussion. The serum antibody response of individuals infected with 1 of 3 chlamydial strains was studied by the C'F and IFA methods. Table II shows some divergence between antibody titers by C'F and IFA for several of the sera studied. The C'F antibodies were often detected 1–2 weeks earlier than the IFA-reactive antibodies (Fig. 1). Peak titers as determined by either method occurred in some volunteers about the fourth week following experimental inoculation and perhaps earlier in others (*e.g.*, volunteer I-7).

We reported earlier that both C'F- and IFA-reactive antichlamydial (TRIC) antibodies, in early and late sera, were IgG immunoglobulins (12). Therefore, it was of interest to determine whether the 2 techniques measure the same antibodies, with the C'F being more sensitive, or whether the IFA technique measures antibodies distinct from those detectable by C'F. The latter possibility was supported by results in some volunteers who, following infection with IC Cal 3, produced antibodies demonstrable by C'F, but who failed to produce any antibodies detectable by IFA throughout the observation periods of 6 and 36 weeks postinfection (Fig. 1, volunteers I-7 and IV-15). This finding strongly suggested that C'F-reactive antibodies are produced in response to antigens which are different from those eliciting the production of IFA-reactive antibodies.

Cross-absorption studies provided further evidence that the C'F- and IFA-reactive antibodies are indeed distinct populations with C'F antibodies carrying the group-reactivity and IFA-reactive antibodies carrying the strain-specific reactivity. The C'F antibody titers were reduced to a similar extent by the absorption of serum with either Bio-Gel or fluorocarbon-purified antigens from IC Cal 3, IC Cal 8, or ASGH.

Nichols and McComb (3), Hanna and Bernkopf (4), Katzenelson and Bernkopf (6), and Nichols *et al.* (13) demonstrated trachoma group antigens by immunofluorescence. Part of the fluorescence obtained in the present study may have been due to group antigens. When antisera against IC Cal 3 or IC Cal 8 were absorbed with ASGH antigens, slight but significant reductions in IFA titers against IC Cal 3 or IC Cal 8 were obtained (Table III). However, absorption of any antiserum with the homologous antigen always reduced IFA titers much more than absorption with heterologous antigens. This indicates that much of the antibody reactive in IFA is strain-specific.

Each absorbing dose of Bio-Gel- or fluorocarbon-purified antigen contained the same number of particles, but fluorocarbon-purified antigens removed more IFA antibodies than did the Bio-Gel-purified preparations. This suggests that fluorocarbon-purified antigens have more antigenic determinants available on their surface for combination with IFA-reactive antibodies than do Bio-Gel-purified antigens. The absorption studies further indicate that the group-reactive (C'F) antigens are probably located on the surface of TRIC particles because intact particles purified by Bio-Gel removed most of the antibodies reactive by the C'F test.

Although limited in scope, these cross-absorption studies suggest that the IC Cal 3, IC Cal 8, and ASGH strains of TRIC agents share a common antigen(s). Inclusion conjunctivitis strains IC Cal 3 and IC Cal 8 are clearly more closely related to each other than to the ASGH (trachoma) strain. This is in agreement with the reports of Hanna and Bernkopf (4) and McComb and Bell (14), who found IC Cal 3 to be distinct from ASGH by immunofluorescence. It is not known whether the antigenic variations observed among these 3 strains represent a qualitative or a quantitative difference in antigen content, but the results reported here

suggest that the group-reactive antigens are more exposed on the surface of the particles whereas the strain-specific antigens are probably masked in their native form. Further absorption studies employing a greater variety of chlamydial strains and antisera are in progress.

Summary. The serum antibody response in volunteers experimentally infected with 1 of 2 inclusion conjunctivitis strains (IC Cal 3 or IC Cal 8) was studied by the complement fixation (C'F) and indirect fluorescent antibody (IFA) methods. In some sera the C'F-reactive antibodies were detected 1–2 weeks earlier than the IFA-reactive antibodies and peak titers, by either test, were attained about 4 weeks after infection.

Cross-absorption experiments revealed that the C'F and IFA methods detect 2 distinct populations of antibodies apparently produced in response to different antigens. The C'F antibodies are principally group-reactive while the IFA-reactive antibodies are, at least in part, strain-specific. The group antigen(s) are probably located on the surface of TRIC particles since most of the group antibodies can be absorbed by Bio-Gel-purified antigens of homologous and heterologous strains used. The strain-specific antigen(s) are detectable on the surface but may be partially masked in the native state. Fluorocarbon treatment markedly increases their availability to antibody and absorption of sera with fluorocarbon-treated homologous antigen permits complete removal of "strain-specific" antibodies.

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