STUDIES ON THE SPECIFICITY OF THE DETECTION OF BOUND COMPLEMENT BY MEANS OF FLUORESCEIN-LABELED ANTICOMPLEMENT

TRANSLATION NO.

741

FEBRUARY 1963

U.S. ARMY BIOLOGICAL LABORATORIES
FORT DETRICK, FREDERICK, MARYLAND

CCHL: FDR-3742 (T-37-1)
JPRS: R-2990-D

26 February 1963

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Translated for:

U. S. CHEMICAL CORPS BIOLOGICAL LABORATORIES
Ft. Detrick, Md.
By:

U. S. DEPARTMENT OF COMMERCE
OFFICE OF TECHNICAL SERVICES
JOINT PUBLICATIONS RESEARCH SERVICE
Building T-30
Ohio Drive and Independence Ave., S.W.
Washington 25, D. C.

STUDIES ON THE SPECIFICITY OF THE DETECTION OF BOUND COMPLEMENT BY MEANS OF FLUORESCEIN-LABELED ANTICOMPLEMENT*

by

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Zeitschrift f. Hygiene.
147 (1961), pp 434-446.

The detection of bound complement by means of fluorescein-labeled anticomplement has led to new findings both in the field of immunopathology and virology. Klein and Burkholder (6) have detected, by means of their published method, immune precipitates in the glomerula of the rat in experimental kidney anaphylaxis. The assumption is warranted that through these findings certain possibilities have been opened up toward the clarification of the pathogenesis of the human nephritis syndrome. In the period following the above discovery Strauss et al (14) found in the sera of patients with myasthenia gravis a complement-binding globulin fraction which reacted with the muscle tissue of the patients and which could be represented fluorescence-optically. Vogt and Kochem (15) called the attention to the fact that amyloid contains complement-binding complexes which can be made visible by means of fluorescein-labeled anticomplement. In the rheumatically altered human heart muscle Klein and Langer (quoted in Klein [5]) have demonstrated fluorescence-optically the existence of a complement-binding process on complexes localized exclusively in the Aschoff bodies. On the basis of these findings theoretical concepts in the sense of immune reactions regarding the pathogenesis of myasthenia gravis, amyloidosis and rheumatic fever seem to have an experimental foundation. Muller (11) was able, with the sera/gonorrheal patients, to perform a cardiolipid-complement-binding

*Study undertaken with the support of the German Research Community (Deutsche Forschungsgemeinschaft) and the German Association for the Fight Against Infantile Paralysis (Deutsche Vereinigung zur Bekampfung der Kinderlahmung).

reaction in the muscle cells of bovine heart, and to detect the reaction fluorescence-optically by means of labeled anticomplement. Finally Ricken (13) has shown by means of a fluorescein-labeled complement-homologous antiserum that in the region of structures described as Pneumocystis carinii in the lungs of infants with interstitial plasma-cellular pneumonia a complement-binding process takes place when the lung section is first treated with the serum of infants who have survived the disease.

Using the same method it was possible to prepare virus-antibody complexes in virus-infected monkey-kidney tissue cultures treated with virus-homologous antibodies. By diluting the respective immune serum it was possible to detect complement-binding antibodies in enterovirus antisera in a titer which escapes the usual methods employed for the detection of complement-binding antibodies (Müller and Klein, 12). As it was possible to show, the procedure is also suitable for the typing of freshly isolated enteroviruses. The advantages when compared with the customary typing procedure in the virus-neutralisation test and the sandwich-technique of Coons et al (1) have been recently discussed in another article (9). A further development of the method, in which one works with unlabeled rabbit anticomplement serum and a fluorescein-labeled anti-rabbit-globulin serum, was indispensable for the optimal evaluation of anticomplement charges for the fluorescence-complement-binding reaction. This indirect method offers advantages in the detection of complement binding to enterovirus-antibody complexes when one works with a deficit in any part of the experimental system (8, 10).

In the following presentation we shall speak generally of "anti-complement." This anticomplement serum is produced by the immunisation of rabbitts with complement bound to antigen-antibody complexes (cf. Klein and Colli [7]). The antibody spectrum of typical anticomplement sera is a narrow one. It is supposedly impossible to produce in this manner immune sera whose antibodies are directed exclusively versus the complement or its components. Essentially it seems that the complement-homologous antibodies exhibit higher titers than those which react with other fractions of the guinea-pig serum. In the working up of immune sera for fluorescein labeling a portion of the antibody is lost. When the complement-homologous antibodies are present in the orginal serum in a considerably higher titer than the complement-heterologous ones, then it is possible for a reagent specific to the complement to be present in the fluorescein-labeled anticomplement.

Already Coons et al (1) have pointed out that the indirect process of fluorescence-serological antigen detection, compared with the direct method, may be burdened with unspecific reaction outcomes. Under certain experimental conditions reactions may take place between the cell substrate and the fluorescein-labeled antibodies: reactions directed not versus the antigen but versus the cell as antigen carrier. This point of view holds true to an even greater extent for the fluorescence-serological preparation of bound

complement, since the tissue section or tissue culture is covered, in succession, by at least three different sera. The possibility that there will take place, between serum constituents and the tissue or tissue parts (nucleus, cytoplasm), bindings simulating a fixation of complement to antigen-antibody complexes, increases with the number of sera necessary for the test. Such reactions, unspecific in the sense of the test, can only be recognized if one employs, contemporaneously with the experiment, a control system corresponding to the requirements.

If an anticomplement serum regularly contained only such antibodies that are directed toward the complement or its components, then in the case of the fluorescence-complement-binding reaction, reaction outcomes, unspecific in regard to the detection of bound complement, could occur only to the extent that they do in the case of Coons' indirect procedure for the detection of antigen. Recently, however, we have had the experience that, depending on the anticomplement charge employed, it is possible, in addition to a specific staining of the antigen-antibody-complement complex, for a fluorescence of the cells, particularly of cell nuclei, to occur independently of the specific staining. The independence of this reaction from immune aggregates could be recognized by the fact that it took place also when the antigen-homologous antibody was omitted. Further, this reaction took place when chelated or decomplemented guinea-pig serum was employed. Reactions of this type between the cell system as antigen carrier and components of the fluorescein-labeled sera impede especially the recognition of enterovirus-antibody complexes in the monkey kidney-tissue culture; they can even make this recognition impossible.

We have endeavored to look for and eliminate the causes of these fluorescence reactions unspecific in regard to the complement binding to enterovirus-antibody complexes, occurring on employment of individual anticomplement charges. We shall report on our findings obtained in these experiments in the present paper.

Materials and Methods

1. Antigens. The white of hens' eggs was stored as a dry preparation and placed on the microscopic slide in 7.5% gelatine (in physiological saline solution) and air-dried.

In the same manner a culture of Bact. proteus OX 19 from agar plates was suspended in physiological saline solution, imbedded in 7.5% gelatine and placed on the microscopic slide.

For virus antigens in the monkey kidney-tissue cultures the following laboratory strains were used: Poliovirus, Type I (Mahoney) with a $TCID_{50}$ of $10^{7.0}$/0.1 ml; Poliovirus Type II (MEF_1) with a $TCID_{50}$ of $10^{6.0}$/0.1 ml; Poliovirus Type III (Saukett) with a $TCID_{50}$ of $10^{6.2}$/0.1 ml; ECHO Virus, Type 9 (V 281), with a $TCID_{50}$ of $10^{6.0}$/0.1 ml; and Coxsackie virus, Type B 3, with a $TCID_{50}$ of $10^{6.6}$/0.1 ml.

2. Immune Sera. The anti-hens'-egg white serum (Anti-Huhnereiklarserum) was obtained by the method of Klein and Colli (7) through immunization of rabbits*. The precipitating antibody titer of this serum amounted to 1:120,000.

In the case of the anti-Bact. proteus OX 19 serum a commercial preparation was used having an agglutinating antibody titer of 1:800.

The sera with enterovirus antibodies were produced partly in our laboratory through immunization of rhesus monkeys, and partly at Behringwerken A.G., the latter being rabbit immune sera. The neutralizing antibody titer at about 100 $TCID_{50}$ amounted, in the case of anti-polio-I serum (of rabbit) to 1:500; in the case of anti-polio-II-serum (of rabbit) to 1:1000; in the case of anti-polio-III serum (of rabbit) to 1:100; in the case of anti-ECHO-9 serum (of monkey) to 1:1280; and in the case of anti-Coxsackie B 3 serum (of monkey) to 1:640. The determination of the neutralizing activity of the sera was carried out in our laboratory in the usual manner (10).

3. Anticomplement Serum and Anti-Rabbit-Globulin Serum. The preparation and evaluation of these sera have been described elsewhere (7, 10). Anticomplement charge 13/14 was prepared according to immunization scheme A, charge 59/60 according to immunisation scheme B of Klein and Colli (7)*.

4. Technique of the Fluorescence-Complement-Binding Reaction. This as well as the preparation of tissue cultures on microscopic slides has already been described (10). One may refer to this paper. One should take care that the virus-infected tissue-culture cells are protected from drying out, so that the virus is available for the experiment in an active form. In every experiment a control system should be established and used concurrently, as we have pointed out elsewhere (12). In the tables of the present paper only one section from the control system is entered in every case.

5. Fluorescein-Labeling of Immune Sera. For labeling, fluorescein isothiocyanate was used. The procedure of dye coupling to the globulin fraction of sera was carried out under the usual conditions.

6. Absorption Process. The absorption of the guinea-pig sera used as complement donor was carried out by means of a monkey-kidney epithelial cell homogenate at +4°C over 12 hours. In some experiments repeated short-term (30 min) absorptions were carried out at +37°C.

Results

In order to understand the causes of a fluorescence reaction independent of the complement fixation on enterovirus antibody complexes,

* We wish to thank Prof. Dr. Klein for kindly placing the sera at our disposal.

it was necessary to carry out the binding of complement or fluorescein-labeled anticomplement in antigen-antibody systems in which the conditions were clearly visible. The antigen should be present in an indifferent basic substance against which no antibodies are visible either in the complement or in the immune serum. In these model experiments it was necessary to first clarify whether, in addition to the complement, other factors of the guinea-pig serum also enter into binding with antigen-antibody complexes and whether there are present in the anticomplement serum fluorescein-labeled antibodies directed toward these factors. Further, it was examined whether the fixation of the antigen with 96% ethyl alcohol has any harmful influence on the fluorescence reaction of the antigen-antibody-complement-anticomplement complex.

For a model we first employed a hens'-egg-white-anti-hens'-egg-white complex prepared in the manner already described. As may be seen from Table 1, this model aggregate treated with complement and fluorescein-labeled anticomplement exhibited a strong fluorescence, both with unfixed and with alcohol-fixed antigen. The reaction was negative when the complement or antibody was omitted. A very weak fluorescence took place when chelated complement, that is, one deprived of Ca++ or Mg++ ions was used. This experiment was reproducible in the form shown in every case, independently of the anticomplement charge employed in the case involved.

Table 1. Preparation of a Hens'-Egg-White-Anti-Hens'-Egg-White Complement Complex By Means of Fluorescein-Labeled Anticomplement

Antigen	(2) nacheinander überschichtet mit			(3) Fluorescenzbefund	
	Anti-(4) Hühner-eiklar-Serum (1:8)	C' (1:20)	Anti-C'* (1:2)	(5) unfixiert	(6) alkoholfixiert
(1) Objektträger mit in Gelatine eingebettetem Hühnereiklar	„	aktiv (7)	„	++++	+++
	„	—	„	ø	ø
	„	cheliert (8)	„	(+)	(+)
	—	aktiv	„	ø	ø

1) Miscroscopic Slide with Hens'-Egg White Imbedded in Gelatin 2) Successively Covered with 3) Extent of Fluorescence 4) Anti-Hens'-Egg-White Serum 5) Unfixed 6) Alcohol-Fixed 7) Active 8) Chelated. --- Key to Symbols: C' = Complement from guinea-pig serum; Anti-C' * = Fluorescein-labeled antibody of rabbit homologous with guinea-pig complement. The intensity of the fluorescence reaction is indicated by: ++++ (very strong fluorescence); +++ (strong fluorescence); ++ (weaker but clearly observable fluorescence); + (weak fluorescence); (+) (very weak fluorescence); ø (no fluorescence).

The same observations were obtained when a bacterial antigen (Bact. proteus OX 19) and an antibody homologous with the latter were used. Such an experiment is shown in Table 2. In some experiments with this model complex a strong fluorescence effect could be observed, though not regularly, also in those cases where the homologous immune serum

was omitted. The control with chelated complement, however, regularly turned out negative in such mixtures. Corresponding investigations with agglutination reaction showed that a part of the guinea pigs serving as complement donors possessed antibodies against Bact. proteus OX 19. Thus in these cases the antigen was covered at the same time as the complement with a homologous antibody. In this way a possible source of error in the fluorescence-complement binding reaction was discovered. This consists in the fact that the guinea-pig serum used as complement contains an antigen-homologous antibody. No relationship was found to exist between the reaction outcome and the anticomplement charge.

These model experiments make it appear probable that, when the presence of an antigen-homologous antibody can be excluded, the antigen-antibody complex of guinea-pig serum will be exclusively or at least predominantly bound by complement and that other components of the guinea-pig serum play no decisive role in such cases. These conclusions are justified by the observations that the reaction outcome was independent of the anticomplement charge (preparation scheme A or B) used in each case, and that the controls with decomplemented guinea-pig serum regularly turned out negative. If there were other components besides the complement which deposit on the antigen-antibody complex, then it would be expected that this would be detected, when using decomplemented guinea-pig serum and a fluorescein-labeled anticomplement (scheme B) in the form of a fluorescence-reaction of the complex.

Table 2. Preparation of a Bact. Proteus-OX 19-Anti-Proteus OX 19-Complement Complex by Means of Fluorescein-Labeled Anticomplement

Antigen	(2) nacheinander überschichtet mit			(3) Fluorescenzbefund	
	(4) Anti-B. proteus OX 19-Serum (1:20)	C' (1:20)	Anti-C'* (1:2)	(5) unfixiert	(6) alkoholfixiert
Objektträger mit in Gelatine eingebettetem Bact. proteus OX 19 (1)	„	aktiv (7)	„	++++	++++
	„	—	„	ø	ø
	„	chelliert (8)	„	(+)	(+)
	—	aktiv	„	ø	ø

1) Microscopic Slide with Bact. proteus OX 19 Imbedded in Gelatine. For rest of Key, see Table 1.

The alcohol fixation of the antigen had no influence on the experimental results. We have obtained identical results already earlier in the case of other antigen-antibody complexes (12).

Further investigations were devoted to the question as to whether the specificity of the reaction outcome in regard to the fixation of complement is dependent on the anticomplement charge when the antigen-antibody complex is located in cells which display a cytopathogenic effect due to multiplication of virus. By way of examples two such experiments with different anticomplement charges are shown in Table 3. Anticomplement serum 13/14 was prepared according to immunization scheme A, serum 59/60 according to immunization scheme B.

When a polio-II-anti-polio-II complex in the monkey kidney-tissue culture is successively covered with complement and fluorescein-labeled anticomplement, it appears that on employment of charge 13/14 the fluorescence reaction is virus-specific, while in the case of charge 59/60 a fluorescence effect may be detected also when the virus-homologous antibody has been omitted. From the constellation of results in the control system of the second experiment (charge 59/60) it may be assumed that the cause of fluorescence cannot be a virus-homologous antibody in the complement serum. Apart from the fact that neither complement-binding- nor neutralizing antibodies could be detected in the guinea-pig sera employed, and also that in the case of guinea pigs spontaneous infections with polio viruses are not known, a fluorescence reaction was found to occur also on employment of chelated complement. In this case a complement-binding to virus-antibody complexes is entirely impossible. Since this virus-unspecific fluorescence effect does not take place regularly but only in the case of certain anticomplement charges, the assumption is justified that, with individual anticomplement charges, the binding of complement-independent substances present in the guinea-pig serum to the virus-infected cells is detected.

Table 3. Comparative Investigations For the Preparation of Polio-II-Anti-Polio-II-Complement Complexes in the Tissue Culture with Different Charges of Fluorescein-Labeled Anticomplement

① Objektträger mit Affennierenepithelzellen, infiziert mit	nacheinander überschichtet mit ②			③ Fluorescenzbefund mit Anti-C'*	
	④ Anti-Polio-II-Immunserum von Affen (1:20)	C' (1:10)	Anti-C'* (1:2)	Charge 13/14	Charge 59/60
Polio-Virus, Typ II (MEF_1)	„	aktiv	„	++++	+++
	„	—	„	ø	n. d.
	—	—	„	ø	+
	—	aktiv	„	ø	+++
	„	chelier	„	(+)	++++

1) Slide with Monkey Kidney-Epithelia, Infected with 4) Anti-Polio-II Immune Serum of Monkey (1:20) mit = with. For rest of key, see Table 1.

If this assumption were correct then it ought to be possible to attain the elimination of the fluorescence-optical side effect through the absorption of the guinea-pig serum by means of comminuted tissue-culture cells. Experiments in this regard did in fact lead to a weakening though not a complete elimination of the virus-independent fluorescence.

In further experiments we attempted to clarify the problem as to whether a mixing of the fluorescein-labeled serum with an equal volume of antibody-free human serum would produce the desired result. In order to be able to judge the influence of the anticomplement serum on the reaction result with certainty it was necessary to resort to the indirect method of fluorescence-serological complement-binding detection and at the same time allow the reaction to run its course gradually. Such an experiment is represented in Table 4.

Table 4. Experiments Aiming at the Elimination of the Virus-Independent Fluorescence by Means of Absorption of the Complement by Monkey Kidney Cells, and Mixing the Anti-Rabbit-Globulin Serum with an Equal Part of Polio-Antibody-Free Human Serum

Objektträger mit Affennierenepithelzellen infiziert mit (1)	nacheinander überschichtet mit (2)				Fluorescens-Befund (6)
	Anti-Polio I-Immunserum vom Affen (1:500) (3)	C' AN-absorbiert (1:10) (4)	Anti-C' (1:5)	Anti-Kaninchen-globulinserum* Menschenserum āā (5)	
Poliovirus, Typ I (MAH)	—	—	—	„	ø
	—	—	„	„	(+)
	—	„	„	„	+
	„	„	„	„	++++

1) Slide with Monkey Kidney Epithelial Cells, Infected with 2) Successively Covered with 3) Anti-Polio-I Immune Serum of Monkey (1:500) 4) C' Absorbed by Monkey-Kidney Cells (1:10) 5) Anti-Rabbit-Globulin Serum*, Human Serum āā 6) Intensity of Fluorescence. For symbols, see Table 1.

It was found that fluorescein-labeled anti-rabbit-globulin serum treated with an equal part of human serum does not react, by itself, with the virus-infected cells. Small amounts of substances deposited out of the anticomplement serum on the virus-infected cells; the detectable but very weak fluorescence effect could be practically neglected. When complement previously absorbed by monkey-kidney cells was additionally added to the cells, the virus-independent fluorescence reaction was somewhat more pronounced though still weak. Only when antiserum homologous to the virus was entered in the process did a very strong fluorescence occur, in the region of the cytoplasm of the virus-infected cells. The nuclear area of the cells remained clear. The rest of the control system (cf. [12]) was in order.

Table 5. Investigations With Cross Reactions in the Enterovirus-Antibody System by Means of the Fluorescence-Complement-Binding Reaction.

Objektträger mit Affennierenzellen, infiziert mit (1)	Antiserum				
	vom Kaninchen (2)			vom Affen (3)	
	Polio I 1:50	Polio II 1:100	Polio III 1:10	ECHO 9 1:80	COX B 3 1:50
Polio I	+++	(+)	ø	ø	ø
Polio II	(+)	+++	ø	ø	ø
Polio III	ø	ø	++++	(+)	ø
ECHO 9	ø	ø	(+)	++++	ø
COX B 3	ø	ø	(+)	ø	+++

1) Slide with Monkey Kidney Cells Infected With 2) Of Rabbit 3) Of Monkey For rest of symbols, see Table 1.

Finally it was shown that it is possible to use for the fluorescence-complement-binding reaction, under the given experimental conditions, also such anticomplement charges that possess a broad antibody

spectrum and which, accordingly, do not allow a differentiation between complement-binding to enterovirus-antibody complexes and deposition of complement-independent components on the cell substrate. When one employs for the test complement previously absorbed by monkey-kidney cells and an anticomplement of charge 59/60 treated with an equal part of human serum, then it is possible to carry out a type-specific detection of enterovirus-antibody complexes in the tissue culture, as can be seen in Table 5. These observations confirmed earlier findings obtained with anticomplement charges which contained almost exclusively complement-homologous antibodies.

Discussion

In connection with the fluorescence-complement-binding reaction a new method is described having manifold applications. In this method the detection of bound complement is carried out directly by means of a fluorescein-labeled anticomplement. It appeared indicated to carry out experiments on the specificity of the procedure in regard to the preparation of complement. These experiments were motivated by the observation of unspecific reaction outcomes, that is, fluorescence effects, in systems in which antigen-antibody complexes were not present. Two questions stood in the foreground of interest: 1. What material present in the guinea-pig serum employed as complement deposits on the antigen-antibody complexes and is detected by means of fluorescein-labeled anticomplement? and 2. What is the significance of the antibody spectrum of the anticomplement serum when the complement binding is to be detected on antigen-antibody complexes which are present in cytopathogenic, that is, morphologically altered cells?

In attempting to answer experimentally the first of the above two questions, it was necessary to start out with as uncomplicated experimental conditions as possible. Antigen-antibody aggregates had to be present in a "naked" form, so that reactions of the complement- and anticomplement serum with accompanying substances could be ruled out with certainty. In addition it was decided to use in these experiments, besides anticomplement sera containing only complement-homologous antibodies, also such anticomplement sera that exhibited a broad antibody spectrum versus the different fractions of the guinea-pig serum.

Previous experiments with enterovirus-antibody complexes in the monkey-kidney tissue culture gave rise to the assumption that individual anticomplement charges not only contain complement-homologous antibodies but also display a considerably broader antibody spectrum. This assumption found a support in the investigations with immune electrophoresis (4). This holds true especially for anticomplement charge 59/60, with which a part of our experiments was carried out. According to the immune-electrophoretic findings this charge is better denoted by the term anti-guinea-pig serum. It nevertheless differs from the anti-guinea-pig-globulin sera of Goldwasser and Shepard (2) by its higher complement-homologous antibody content, as could be demonstrated by precipitation results.

Studies by means of the fluorescence-complement-binding reaction in model complexes (hens'-egg-white/anti-hens'-egg-white; Bact. proteus OX 19/anti-Bact. proteus OX 19) showed that complement binds to the immune aggregates after the latter have been brought together with guinea-pig serum, and that this complement can be detected by means of fluorescein-labeled anticomplement. The fluorescence reaction with a complement-homologous antibody was just as clear as with an anti-guinea-pig serum. Had there been other components instead of the complement fixed to the antigen-antibody complexes, then the reaction with complement-homologous antiserum would have had to turn out negative. Nevertheless, the simultaneous occurrence of complement binding and fixation of complement-independent substances of the guinea-pig serum cannot be ruled out in this manner with absolute certainty. There is, however, an experimental indication that complement-independent substances play no role in these antigen-antibody systems in that control tests with decomplemented complement and anticomplement charges having a broad antobody spectrum regularly turned out negative.

The fixation of the antigen with 96% alcohol was found to have no importance for the fluorescence result. The experiments with the bacterial immune aggregate showed that antigen-homologous antibodies in the guinea-pig serum (the latter serving as complement) may lead to errors which can be recognized by means of a careful control system.

The rest of the experiments were related to virus-infected and correspondingly cytopathologically altered tissue-culture cells treated successively with virus-homologous antibody, complement and fluorescein-labeled anticomplement serum possessing different antibody spectra. It could be shown in comparative experiments with different anticomplement charges that on employment of some charges a fluorescence-optically detectable effect may occur in virus-infected cells also in the case when the virus-homologous antibody is omitted or substituted by a heterologous antibody. No such reaction ever occurred in non-infected cells when the latter were covered, successively, with virus antibody, complement and fluorescein-labeled anticomplement. The presence of a virus-homologous antibody in the guinea-pig serum (complement) could be ruled out. From these observations we would like to conclude that the cytopathogenically altered cell behaves differently toward the guinea-pig serum than toward the intact cell. We have already mentioned elsewhere that the virus-infected cell apparently becomes permeable to substances, present in the guinea-pig serum, which do not belong to the complement, deposit unspecifically on components of the nucleus and cytoplasm, and cannot be eliminated through washings carried out at intervals (9).

These virus-independent fluorescence effects in the cytopathologically altered tissue-culture cells, effects which can be detected through the control system and which occur on employment of anticomplement sera with very broad antibody spectra, constitute an apparently unsupportable disturbing factor. For immunological studies with highly

purified complement reagents it is assumed that one may use preparations having a broad antibody spectrum. One can only conclude that the complement-homologous antibody possesses a sufficiently high titer. How such anticomplement charges act in tissue sections during immunohistological studies carried out with antigen-antibody complexes cannot be decided on the basis of our experiments. For the fluorescence-complement-binding reaction in cytopathogenically altered tissue-culture cells one could, however, assume the position that only such anticomplement preparations should be employed that exhibit - in any event, according to the fluorescein labeling - a narrow antibody spectrum, whereby the antibodies would be limited to complement homologues. As could be shown the risk of unspecific reaction outcomes is high when -as proposed or carried out by some authors (2, 3)- one works with anti-guinea-pig-globulin serum and wants to obtain data regarding the binding of complement to virus antibody complexes without adequate specificity controls.

It further experiments we wanted to determine how the binding of complement-independent material present in the guinea-pig serum to the virus-infected monkey-kidney cells may be prevented. It was obvious to think of an absorption of the anticomplement serum prior to the fluorescein labeling by means of decomplemented guinea-pig serum. Such experiments, however, must work out erroneously, since different components of the guinea-pig serum, especially those of the β-globulin fraction, cross-react with the complement components. Hence complement-homologous antibodies would be precipitated by factors belonging to the complement. One may consider, as a further possibility, the absorption of guinea-pig serum by means of a homogenate of tissue-culture cells. Experiments relating to this method, however, showed that in this way a full suppression of the unspecific staining of tissue-culture cells is not possible. Only when one resorts to the addition of an equal part of antibody-free human serum to the fluorescein-labeled antiserum does the production of a fluorescence reaction independent of virus-antibody complexes fail to take place. However, we do not have any experimental clarification of this phenomenon which, in a similar situation, has already been confirmed in other laboratories (4).

By using guinea-pig serum previously absorbed by means of monkey-kidney cell homogenate as complement, and an anti-guinea-pig serum treated with an equal part of human serum, it was possible to produce a virus-ytpe-specific fluorescence reaction. In this way it was possible to verify and enlarge earlier findings which have already been described and which were obtained with complement-homologous anticomplement charges (9).

Summary

Using model complexes (hens'-egg-white/anti-hens'-egg-white; Bact. proteus OX 19/Anti-Bact. proteus OX 19) it was first shown that, in the case of the employment of fluorescein-labeled anticomplement in the fluorescence-complement-building reaction the fixation of complement to the antigen-antibody complexes is detected. Other factors of the guinea-pig

serum. against which antibodies may exist in the anticomplement serum, play no role in this reaction. The fixation of the antigen with 96% ethyl alcohol has no influence on the fluorescence effect.

In the virus-infected tissue culture with cytopathogenic effect of the cells there takes place, in addition to a complement binding to virus-antibody complexes, an unspecific deposition of complement-independent components of the guinea-pig serum on nuclear- and plasma constituents of the tissue cells which cannot be eliminated by means of a washing of the cells. The causes of this deposition detected with anticomplement charges having a broad antibody spectrum, and which [deposition] simulates a complement binding to virus-antibody complexes, are discussed.

The main reason is seen to lie in the fact that anticomplement charges of rabbits which had been strongly immunized exhibit a very broad antibody spectrum. In order to eliminate these unspecific reaction results it is not sufficient to absorb the complement-containing guinea-pig serum by means of homogenates of the tissue culture. It has nevertheless proved to be effective to treat the fluorescein-labeled antiserum simultaneously with an equal part of antibody-free human serum. Under these experimental conditions it is possible to use also anticomplement preparations with a broad antibody spectrum for the fluorescence-serological detection of complement binding to enterovirus-antibody complexes. The results exhibit the same type specificity as when employing anticomplement that contains exclusively complement-homologous antibodies.

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(4) Klein, P: Private Communication.

(5) Klein, P: Complement (C') and its Reaction with Antigen-Antibody Complexes (In Press).

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(7) Klein and Colli: Preparation and Evaluation of "Anticomplement."

(8) Muller, F: Direct and Indirect Detection of Complement Binding to Virus-Antibody Complexes in Tissue Culture.

(9) Muller,F: Fluorescence-Serological Detection of Complement Binding as a Virological-Diagnostic Method in Enetrovirus Infections.

(10) Muller, F.: Quantitative Investigations on the Direct and Indirect Fluorescence-Serological Detection of Complement Binding to Enterovirus-Antibody Complexes (In Press).

(11) Muller: Fluorescence-Serological Studies on Complement Binding to Antigen-Antibody Complexes (In Press).

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(15) Vogt, A., and H.G. Kochem: Histo-Serological Investigations with Fluorescein-Labeled Anticomplement. Detection of Complement-Binding Substances in Amyloid.]