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Antigen-Antibody Interactions *in vitro*: II. The Non-Neutralizing Antibodies are by far the Most Potent Virus Inactivators

Viggo Bitsch*

Abstract

Inactivation of the virus in a conventional neutralization test was in early comprehensive studies found to be a bifactorial process, consisting of a prompt and short-lasting reaction originally termed "over-neutralization" and an enduring but slowly progressing reaction of first order. The reaction of first order follows the lines of the formula of the regular antigen-antibody interactions not including aggregation. These lines could be recognized because antigens and their antibodies are bound irreversibly under physiological conditions. Tests for demonstration of virus and antibodies of very high sensitivity and specificity have been elaborated on basis of these reaction lines and used in diagnosing, controlling and eradicating viral infections in the veterinary field for a long time. The early and rapid "over-neutralization" reaction could later be concluded to be neutralization by aggregation of viruses.

The non-neutralizing antibodies inactivate viruses by aggregation of the agents. *In-vitro* studies have demonstrated an extremely high virus-inactivating potency of these antibodies. The principal characteristics of virus aggregation are the following: 1) the antibodies to various antigenic determinants aggregate virions synergistically and rapidly, 2) the virus-inactivating aggregation by the polymerized isotypes of antibodies is greatly enlarged by their polyvalency, and 3) the complement component C1q will promptly attach to antibodies sensitized by being bound to their antigenic determinant on a virus and inactivate such virus-antibody complexes by including them in aggregates. In complement-enriched neutralization tests, C1q will promptly aggregate antigen-antibody complexes formed almost immediately and with increasing reaction times aggregate the test virus following the first-order binding of non-neutralizing antibodies to their antigenic determinants, inactivating viruses with the same rate as neutralizing antibodies.

In a herpesvirus complement-enriched neutralization test, the titers of reacting non-neutralizing IgG antibodies in the highest concentration was found to be approx. 8 times higher than that of the neutralizing antibodies. The total concentration in blood of non-neutralizing antibodies in blood by far exceeds that of the neutralizing ones, being largely proportional to the number of antigenic determinants on a virion. The neutralizing capability of the non-neutralizing antibodies by aggregation, most pronounced in cooperation with the C1q complement component, implies that the non-neutralizing antibodies have a much greater neutralizing potency than neutralizing antibodies. One non-neutralizing antibody bound to an antigenic determinant will result in almost immediate inactivation of the virus due to aggregation created by the C1q component.

Affiliation:
The State Veterinary Serum Laboratory, Copenhagen and The Department of Cattle Diseases, The Danish Dairy Board, Aarhus, Denmark

***Corresponding author:** Viggo Bitsch.

Email: viggo.bitsch@gmail.com

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The formation of the pentameric and decavalent IgM antibodies is the first humoral immune response after infection and the virus-inactivating potential of these antibodies is enormous. A significant neutralizing IgM antibody response could be detected in blood by a complement-enriched neutralization test 4 days after nasal infection and samples taken after 8 to 15 days were positive in dilutions 1:10.000 or higher. The IgM antibodies, in cooperation with the C1q component of complement, must be considered the ultimate virus inactivators and of almost unimaginable importance in the defeat of infections.

Unidentified specific forces attracting 1) the binding sites of antibodies and their specific determinants on viruses, and 2) the binding sites of the C1q component and the Fc fragment of sensitized antibodies bound to their antigenic determinants, are concluded to be one reason for the prompt reaction, which is characteristic of the virus aggregation process.

Further investigations into the effector mechanisms determining 1) the specificity and attractive binding of the antibodies to their antigenic determinants and 2) the reaction between the complement component C1q and sensitized antibodies are urgently needed.

Keywords: Virus neutralization; Non-neutralizing antibodies; Inactivation by aggregation.

Article Highlights

A description of the characteristics of the complex virus-aggregation reactions by specific antibodies. A demonstration of the extensive virus-inactivating potency of the non-neutralizing antibodies through aggregation of the agents.

1. Introduction

The significance of the various antigen-antibody interactions may be difficult to assess. However, it appears evident from *in-vitro* investigations that the importance of the non-neutralizing antibodies in the combatting of infectious diseases has been greatly underestimated. This is the main reason for this analytical review.

Definitions

- *Neutralizing and non-neutralizing antibodies:* neutralizing antibodies inactivate viruses by being bound to their antigenic determinant on the virion, while non-neutralizing antibodies are unable to neutralize the virus simply by being attached to their antigenic determinants. An antigenic neutralization determinant is the specific antigen of a neutralizing antibody.
- *The reacting antibodies of the highest concentration* determine the titer measured in an antibody test. This titer is also a measure of the *test sensitivity*.

2. Basic Antigen-Antibody Interactions

Figuratively, the IgG antibody molecule can be considered the basic antibody unit. It is Y-shaped with two identical Fab fragments, each with an antigen-binding site at the free end, and one Fc fragment. This basic unit is monomeric and divalent. The secretory IgA antibody is, at its sites of function extracellularly, composed of two basic units and is dimeric and tetravalent, and the IgM antibody, composed of 5 basic units, is pentameric and decavalent. Specific isotype antibodies directed towards different antigenic determinants on a virion react synergistically in the aggregation process, and the aggregating potency of tetra- and decavalent antibodies will be far greater than that of the IgG antibody.

Early investigations of antigen-antibody interactions *in vitro* demonstrated a constant rate of virus neutralization in a virus-antibody mixture when virus titers were recorded logarithmically (*Andrews and Elford* 1933) [1], and a direct proportionality between the neutralization rate and the antibody concentration (*Burnet et al.* 1937) [2]. The first-mentioned authors also hypothesized a so-called *percentage law* implying that the neutralization rate is independent of the antigen concentration.

Despite these investigations, it became widely acknowledged that the antigen-antibody binding reaction is reversible under physiological conditions, most probably because no substantial improvement of the test sensitivity could be obtained in neutralization tests by increasing the reaction time over the first couple of hours. Reversibility implicates that the reaction will lead to a state of equilibrium and that no further improvement of the test sensitivity is possible after the alleged equilibrium.

In a comprehensive herpesvirus study, however, the significance of four variables of the reaction rate in a neutralization test, i.e., the virus and antibody concentrations and the reaction time and temperature, was evaluated. It was documented that the reaction is bifactorial, consisting of a rapid but short-lasting "over-neutralization" reaction and an enduring and slowly progressing first-order neutralization reaction (*Bitsch* 1978) [3]. The lines of this first-order antibody reaction identified were crucial for the capability to elaborate antigen-antibody tests of high sensitivity.

Figure 1 shows neutralization titers in serum with short reaction times, but the expected first-order progression with a log-log slope coefficient of 1 for the antibody-titer/reaction-time line is not observed until after reaction for more than 2-3 hours at 37 °C (*Figure 2*). The early extraordinary reaction concluded to be a regular condition was termed the "over-neutralization" reaction because this reaction was more pronounced than expected from the perception of a first-order reaction.

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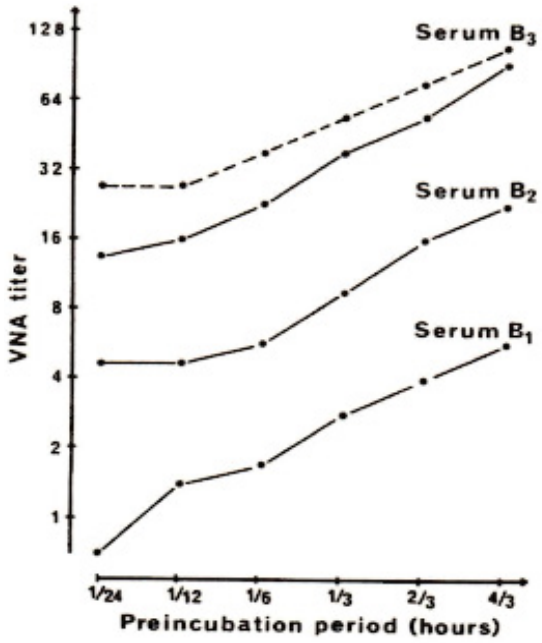

Figure 1: Kinetics of virus neutralization at 37 °C for 3 late-infection (IgG) sera in the dilution series of neutralization tests with very short reaction periods. From *Bitsch* 1978 [3].

Virus: BoHV-1. VNA: virus-neutralizing antibody. Preincubation: reaction time. After reaction, the virus-serum mixtures were inoculated onto cultures with maintenance medium involving an immediate 1:10 dilution of the mixtures. The dashed line shows the results from a testing of Serum B_3 with inoculation of the virus-antibody mixtures onto cultures without maintenance medium. It should be noted that reaction times are usually plotted non-logarithmically. If they had not been shown logarithmically, the slope of the reaction lines would have been less steep. Log-log reaction lines of a first-order reaction by antibodies would have been linear with a slope coefficient of 1.

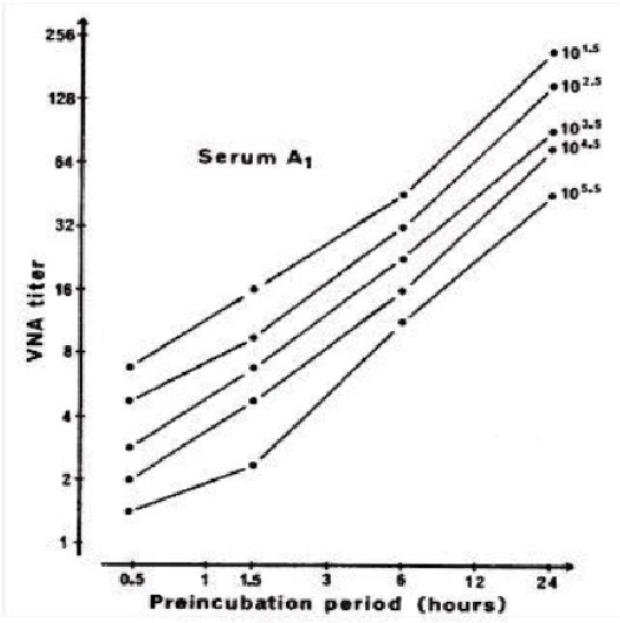

Figure 2: Kinetics of virus neutralization at 37 °C for a late-infection (IgG) serum sample in the dilution series of neutralization tests with extended reaction periods and varying virus concentrations. From *Bitsch* 1978 [3].

Virus: BoHV-1. VNA: virus-neutralizing antibody. Preincubation period: reaction time.
The neutralization lines are identical, varying only with the virus concentration. With reaction times exceeding 2 to 3 hours, the log-log curves are linear with a slope coefficient of 1, which is characteristic of a first-order antibody-binding reaction. Other measurements in this study documented a strictly log-log linear relationship with a slope coefficient of 1 for reactions up to at least 48 hours at 37 °C, 4 days at 26 °C, and 8 days at 15 and 4 °C.

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The early reaction hiding the slow first-order antibody neutralization could not immediately be explained. However, the observation by *Brioen et al*. 1983 [5] that virus particles aggregated by antibodies were rendered non-infectious, revealed a new method of virus-inactivation in addition to the one known for the neutralizing antibodies, cf. *Definitions*. The early and almost explosive, short-lasting "over-neutralization" was concluded to be neutralization due to the aggregation of virions by predominantly non-neutralizing antibodies (*Bitsch* 2017) [4]. Late-infection antibody samples were used in the 1978 study, so the early over-neutralization observed was virus-aggregation caused synergistically by the different divalent IgG antibodies, whereas the first-order neutralization seen with extended reaction times was the slowly progressing effect by the neutralizing antibodies being bound firmly and monovalently to their antigenic neutralization determinant on the virions.

$$k_{st} = \frac{[Ab][Ag]^q}{T}$$

The formula for the regular *in-vitro* antigen-antibody interactions, was presented [3]. In this formula, k_{st} is the standard reaction rate factor, Ab and Ag are antibody and antigen titers, T is the reaction time, and q is a particular co-determiner of the reaction rate. This factor q is temperature-dependent and was found to be approx. 0.15 at 37 °C but 0.24 at 4 °C. Being temperature-dependent but independent of the antigen and the reacting antibody concentrations, this factor appears to be a fundamental characteristic of the antigen-antibody binding reaction. According to the percentage law [1], the factor q should have the value of 0 and correspondingly $[Ag]^q$ in the formula the value of 1, so that hypothetical law was found invalid [3,4]. Three of the four variables determining the reaction rate factor are seen directly in the formula and the fourth, the reaction temperature, is shown indirectly by the factor q being temperature-dependent.

The formula shows that the regular antibody reaction in a neutralization test will be of first order, implying that the titer recorded and the test sensitivity will be proportional to the reaction time. For a herpesvirus neutralization test, the increase of the reaction time from 1 to 24 hours raised titers and the test sensitivity, not by a factor of 24 as indicated by the formula, but by a factor of 16-18 [4] because a remaining aggregation reaction was measured after 1 hour of reaction, cf. *Figure 2*. Other investigations in the 1978 study [3] documented a continuing log-log linear relationship for the antibody titer and reaction time with a slope coefficient of 1 for reactions up to at least 48 hours at 37 °C, 4 days at 26 °C, and 8 days at 15 and 4 °C. In a test demonstrating antigen, the reaction will be exponential and depending on the value of q. In an antigen ELISA, for example, considerable improvements in the test sensitivity will be achieved with extended reaction times when values of q are below 1. However, both antigen and antibody ELISAs can be modified and performed with a short reaction time and very high sensitivity by incorporating appropriate aggregation reactions [4].

The study of the complement-dependent neutralization by *Bitsch and Eskildsen* 1982 [6] showed that also non-neutralizing antibodies were connected firmly and irreversibly to their antigenic determinants, following the lines of the formula above. Figure 3 shows the progression of the binding of neutralizing and non-neutralizing IgM antibodies to the virus in dilution series of an early convalescent-phase serum sample. The C1q component of complement was used to visualize the binding by the non-neutralizing antibodies. After the addition of complement, here especially after either 5, 11, or 23 hours of reaction, the results documented a preceding, rigidly continuous first-order reaction by the non-neutralizing IgM antibodies (2-2, 3-3, and 4 in Figure 3) with a log-log slope coefficient of 1 for the reaction lines, indicating irreversible bindings.

Although the *virus titer* and the *virus concentration* ordinarily are congruent terms, the *antibody titer* and the *antibody concentration* are widely different because of usually a high number of antigenic determinants on a virion giving rise to the formation of a variety of specific antibodies. The titer of an antibody sample indicating the test sensitivity is determined by the reacting antibodies being present in the highest concentration and not by the total concentration of reacting antibodies, cf. *Definitions.*

Antigen and antibody tests of very high sensitivity could be elaborated based on the demonstrated regular lines of antigen-antibody reactions. This cleared the way for the pioneering eradication in Denmark over 2-3 decades of the three most costly, widespread respiratory viral infections in swine and cattle.

The main results from the study of the complement-dependent neutralization reaction [6] are presented below. An analytical review of antigen-antibody interactions *in vitro* was published in 2017 [4].

3. The Neutralization Characteristics of Non-Neutralizing Antibodies

Non-neutralizing antibodies inactivate the specific virus by aggregating the virus particles [5,4]. The aggregation reaction directly by di- or polyvalent antibodies without interference from complement has already been described in *Section 2.*

The hexavalent complement component C1q will bind to the Fc region of any antibody sensitized by being bound to its antigenic determinant on a virus, and the attachment of the C1q molecule to another sensitized antibody will include the virus in non-infectious virus-antibody aggregates.

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In conventional neutralization tests, where complement in test samples is routinely inactivated by heating, no neutralization by the action of complement will be seen.

The supplementary neutralization generated by the non-neutralizing antibodies in cooperation with C1q is illustrated in *Figures 3* and *4*. After complement has been added, neutralization by aggregation is practically instantaneous for all virus-antibody complexes formed, but thereafter with increasing reaction periods, it will be of first order, following the first-order binding of non-neutralizing antibodies to their antigenic determinants (*Figure 3*). C1q raises IgG antibody titers by a factor of approx. 8, indicating a considerably

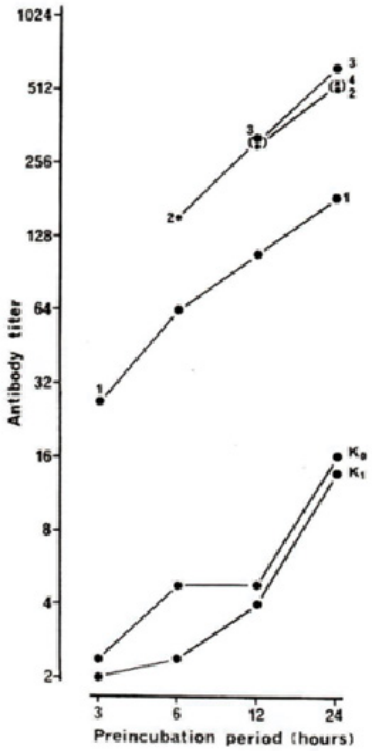

Figure 3: The effect of complement on the progression of neutralization in dilution series of an early convalescent-phase serum sample tested in virus neutralization tests. From *Bitsch and Eskildsen* 1982 [6].

Virus: SuHV-1. Preincubation period: reaction time.

Serum from an experimental pig was collected 13 days after nasal infection. Virus-serum mixtures were incubated at 37 °C, and titers were recorded by inoculation of cultures after reaction for 3, 6, 12, or 24 hours. K_0 and K_1: no complement (K_0) or heat-inactivated complement (K_1) was added at the start of virus-serum incubation. For the measurements 1-1, complement was added at the start of incubation and for the measurements 2-2, 3-3, and 4, complement was added one hour before inoculation of cultures, i.e. after 5, 11, or 23 hours of reaction, respectively.

The log-log slope coefficient of all complement neutralization lines is 1, documenting a first-order, monovalent reaction. An optimal effect of complement is not obtained if it is added at the start of virus-serum incubation (reaction line 1-1).

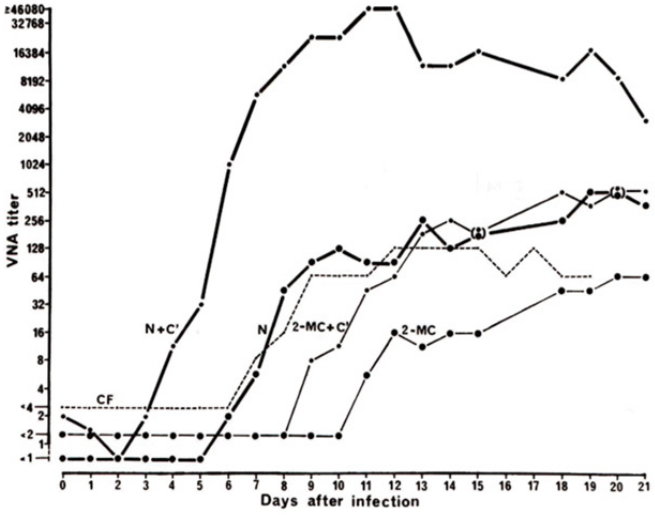

Figure 4: The appearance of non-neutralizing and neutralizing IgM and IgG antibodies in blood during the first 21 days after experimental, nasal herpesvirus infection. From *Bitsch and Eskildsen* 1982 [6].

Virus: SuHV-1. VNA: virus-neutralizing antibody. Tests: the first-order neutralization test and the complement-enriched neutralization test.

The porcine serum samples were heated at 56 °C for 30 min. and tested, either untreated (N) or treated with 2-mercaptoethanol (2-MC), which will inactivate the IgM antibodies, but leave IgG antibodies unchanged. In both cases, the sera were tested with and without the addition of complement (C′). The virus-serum mixtures were incubated at 37 °C for 24 hours and, where used, complement was added after 23 hours of reaction. Results from a complement fixation test are also shown (CF). As expected, the CF titer line follows the one for the titers of the reacting non-neutralizing IgM antibodies in the highest concentration, although at a lower level of sensitivity.

Symbols:
N+C': non-neutralizing IgM antibody titers
N: neutralizing IgM antibody titers
2MC+C': non-neutralizing IgG antibody titers
2MC: neutralizing IgG antibody titers

higher level of the non-neutralizing IgG antibodies in the highest concentration than that of the neutralizing antibodies (*Figure 4*) [4,6]. The improved neutralization caused by IgM antibodies in association with C1q is overwhelming. In *Figure 4*, a significant neutralization reaction by non-neutralizing IgM antibodies in serum is seen 4 days after nasal infection and in samples collected after 8 to 15 days a reaction was measured in serum dilutions 1:10.000 or higher.

The main features of the virus-inactivating aggregation are 1) that various antibodies to antigenic determinants aggregate virions synergistically and rapidly, 2) that the aggregating potential of the polymerized antibody isotypes is greatly enlarged because of their tetra- or decavalency and wider span between antigen-binding sites, and 3) that the complement component C1q promptly aggregates virus-antibody complexes where the Fc fragments have been sensitized by

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the antigen-antibody connection. The aggregation reactions are complex, but a principal characteristic is that the reaction is rapid.

It is worthy of note that one particular effect of the C1q component is that non-neutralizing antibodies are converted into neutralizing ones, first by the almost immediate attachment to a non-neutralizing antibody sensitized by being connected to its antigenic determinant and second by the subsequent inclusion of this virus-antibody complex in aggregates, as illustrated in *Figure 3*. In complement-enriched neutralization tests with extended and increasing reaction times, non-neutralizing antibodies is seen to neutralize the test virus with the same rate as neutralizing antibodies, following the lines of the formula for the regular antigen-antibody interactions.

4. Discussion

One aspect of biomedical research is to disclose how higher animals function and how severe problems have been resolved. Regarding antigen-antibody interactions, one might think that *Nature* had realized that inactivation of infectious agents could be markedly augmented by their aggregation. *Step 1* in the refined defense, after the construction of the Fab fragment of the antibody molecule, would then be the insertion of a second Fab fragment, both with the antigen-binding site at their free end, enabling the various specific antibodies to aggregate target agents synergistically. *Step 2* would be the polymerization of the basic antibody unit into isotypes with a considerably improved aggregating effect because of more binding sites and a wider span between these sites. Finally, *Step 3* would be the introduction of the complement component C1q, a hexavalent aggregator, that would be capable of inactivating viruses that had been coupled with an antibody, neutralizing or non-neutralizing, first by the prompt binding to the Fc fragment of that sensitized antibody molecule and second by the inclusion of such antigen-antibody complexes in aggregates.

As mentioned above, the essential characteristics of virus aggregation by antibodies are that the various antibodies to antigenic determinants aggregate virions synergistically and rapidly, that the tetra- or polyvalency of di- or polymerized antibodies increases the aggregating potency of antibodies significantly, and that C1q rapidly will aggregate virus-antibody complexes. In contrast to the slowly progressing first-order neutralization by neutralizing antibodies seen *in vitro*, aggregation reactions are prompt.

The formation of the pentameric and decavalent IgM antibodies is the first humoral immune response to a viral infection, and most of these antibodies to antigenic determinants on a virus are non-neutralizing, cf. *Figure 4*. Their presence is temporary, largely limited to the acute infection phase, but they appear very early and their concentrations increase rapidly to extremely high levels. Above all, they must be considered to be, particularly in cooperation with the complement component C1q, the ultimate viral aggregators of almost inconceivable importance in the defeat of infectious agents.

In immunology textbooks, the antigen-antibody reaction is chiefly described as being reversible, leading to equilibrium as indicated by the formula Ag + Ab ↔ Ag:Ab. In consequence, it would not be possible to improve the test sensitivity after the achievement of the alleged equilibrium state. The alleged reversibility of virus-antibody bindings under physiological conditions is a hypothesis that has never been documented [4]. As described earlier, cf. *Section 2*, the reaction in a conventional neutralization test is composed of two different reactions, *i.e.*, an early "over-neutralization" reaction being rapid and short-lasting and a strictly first-order reaction being enduring and slowly progressing [3]. The latter could not be distinguished from the first one as a separate reaction type until after a reaction at 37 °C for more than 2-3 hours (*Figure 2*). These conditions, implying that the reaction in neutralization tests with reaction periods up to a couple of hours will be found almost independent of the reaction time, will explain the widely acknowledged, but erroneous concept that antigen-antibody reactions will lead to a state of equilibrium, following the law of mass action (also called the law of chemical equilibrium).

Herpesviruses have been found to possess a high number of different antigenic determinants, but the number of *neutralization* determinants, only one type or very few, appears to be uncertain. Monoclonal antibodies may have been incorrectly identified as neutralizing by authors not realizing the general ability of antibodies to neutralize by aggregation.

Definitions of antibody affinity and avidity are found in immunology textbooks and IgM antibodies are specified as low-affinity antibodies. However, what matters in connection with antigen-antibody reactions *in vitro* is whether the bindings are firm or reversible under physiological conditions. As mentioned above, cf. *Section 2*, no findings so far have indicated reversibility and all stringent analyses have documented firm irreversible bindings under physiological conditions [4].

Because of the many different antigenic determinants on a virus, multiple reactions will occur in virus-antibody mixtures between non-neutralizing antibodies and their antigenic determinants. However, it should be realized that the accumulated reaction measured for several first-order reactions in a neutralization test will appear as a first-order reaction. It should also be noted that if a considerable part of the non-neutralizing antibodies should not react with their antigenic determinants following the lines of a first-order reaction, nor the accumulated reaction will be of first-order.

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The mechanisms of the binding process for 1) antigens and their antibodies and 2) the complement component C1q and the Fc fragment of sensitized antibodies, are extremely important. This issue is closely linked to the matter of specificity. The bindings will be conditioned by a certain sensitization that for antibodies will make only reactions with their specific antigenic determinant possible. The interaction must be biphasic. First, the binding sites will have to be attracted to each other by a magnetism-like force, bringing these sites into position for a union, because without such a force, the small binding sites would not be able to reach each other. The subsequent phase will be the instant firm union of the reactants. A similar attraction and binding relationship must apply for the connection of C1q to sensitized antibodies.

These non-identified attractive forces must be a major reason for the almost instantaneous aggregation observed in antigen-antibody interactions. However, it should be remembered that the early prompt virus aggregation in virus-antibody mixtures of a neutralization test by the various non-neutralizing antibodies without interference from complement is also promoted by their synergistic aggregative action [4]. The early virus-aggregation reaction in an antibody medium without complement depends highly on the antibody concentrations and is readily diluted away in the dilutions of a neutralization test.

New disciplines like biorthogonal chemistry and click chemistry have emerged and in immunobiology, specific bindings between avidin and biotin and between staphylococcus protein A and immunoglobulins have been used in antigen-antibody testings for decades. Although we are coming closer to an understanding, we do not as yet have the knowledge to explain 1) the mechanisms leading to the formation of specific antibodies and their powerful attraction and binding to antigenic determinants, or 2) the sensitization of antigen-bound antibodies and the subsequent prompt attachment to the complement component C1q.

The substantial neutralizing capability of the non-neutralizing antibodies raises the question of how the most efficacious vaccines can be made. Over the past three decades, virus vaccine producers have followed a trend to make sub-unit vaccines giving rise to the formation of especially neutralizing antibodies. *In vitro*, the non-neutralizing antibodies are certainly the most potent inactivators of viruses, they are configured especially to serve aggregation purposes, and their aggregating, virus-inactivating effect is rapid. Although *in-vivo* relations are complicated, these main *in vitro* conditions might also hold extracellularly *in vivo*.

Another aspect is related to the defense against respiratory viruses, which infect individuals on mucous membranes of the respiratory tract, where the dimeric, tetravalent secretory IgA antibodies have been found to predominate. These antibodies are also constructed to favor synergistic inactivation of infectious agents by aggregation. Also complement may play an important defensive role in aggregating virus-antibody complexes on mucous membranes.

There are reasons for the complex and effective virus-inactivating aggregation revealed in *in-vitro* investigations of antigen-antibody interactions. There is an urgent need for further investigations into the effector mechanisms creating the attractive binding forces and determining the specificity in antigen-antibody interactions applied by *Nature* in combatting infectious agents.

Important relations and conclusions recapitulated

- Antibodies, neutralizing and non-neutralizing, are bound firmly and irreversibly to their antigenic determinants under physiological conditions.
- Two reactions occur in a conventional virus neutralization test, i.e., an early almost instantaneous and short-lasting virus aggregation by di- or polyvalent antibodies and an enduring, slowly progressing reaction of first order by neutralizing antibodies, following the lines of the formula for the regular antigen-antibody interactions. Both reactions lead to virus inactivation.
- The principal characteristics of the aggregation reactions are, 1) that di-or polyvalent antibodies aggregate viruses synergistically and rapidly, 2) that aggregation is significantly increased by polymerization of antibodies, and 3) that the C1q component of complement practically instantly will aggregate viruses bound to an antibody. All aggregations are fast, but highly dependent on antibody concentrations.
- A main reason for the extremely rapid aggregation reactions must be a magnetism-like attractive force between 1) specific antibodies and their antigenic determinants on the virion and 2) the C1q component and the Fc fragment of antibodies sensitized by being bound to their antigenic determinants. Also, the synergistic reaction by the various antibodies to different antigenic determinants will advance the aggregation speed.
- One particular effect of C1q is that non-neutralizing antibodies are converted into neutralizing antibodies, inactivating the virus in complement-enriched neutralization tests with extended and increasing reaction times with the same rate as neutralizing antibodies in a conventional neutralization test.
- The pentameric and decavalent IgM antibodies are, especially in close cooperation with the complement component C1q, the ultimate virus aggregators and of huge importance in the combat against viral infections. Their presence is ordinarily limited to the acute infection phase and their concentration in blood is very few days after infection raised to extremely high levels.

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Author's Declaration

Publication involves no conflicts of interest related to persons, institutions, or corporations.

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