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SHORT COMMUNICATION

THE IMMUNODEPRESSIVE EFFECT OF RINDERPEST VIRUS

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SUMMARY

The effect of rinderpest virus on the immune response of rabbits to subsequently administered chicken erythrocytes has been investigated as a means of assessing the effects of this virus on general immunological responsiveness.

A significant depression of the primary response was consistently found in those rabbits which became febrile following viral inoculation. In some cases this inhibitory effect extended to the secondary immune response to the same antigen given approximately 3 weeks after the virus.

The degree of immune depression did not appear to be related to the concentration of virus administered.

These observations are discussed in relation to the pathogenesis of Rinderpest and other myxovirus infections.

INTRODUCTION

The ability of a variety of chemical and biological substances to reduce or suppress immune responses has been intensively investigated within recent years. Infectious agents, particularly viruses, may also exert an inhibitory effect upon immunological mechanisms although this aspect of immunosuppression has received little attention so far. The subject of immunosuppression by virus has recently been reviewed by Salaman (1969) and it is clear that interest in this field has been largely concentrated upon effects mediated by the oncogenic viruses.

It was considered that rinderpest virus might provide a suitable example of a non-oncogenic virus having immunodepressive activity for the following reasons:

(1) It has a selective affinity for lymphocytes and gives rise to very severe changes in lymphoid tissues both in the natural disease in cattle (Maurer *et al.*, 1955) and following experimental infection in rabbits (Fukusho & Nakamura, 1940).

(2) Clinically, the disease in cattle is characterized by gastro-enteric and other symptoms reminiscent of radiation sickness (Scott & Brown, 1961).

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(3) In less acute rinderpest, the activation of latent infections, particularly protozcal, is frequently observed (Curasson, 1932) suggesting a general loss of immunity.

The effect of rinderpest virus on general immunological responsiveness has, therefore, been examined. Evidence is presented of marked inhibitory activity and it is suggested that this may be an important factor in the pathogenesis of the natural disease.

MATERIALS AND METHODS

Rabbits

Rabbits weighing between 1 and 2 kg were bled from the marginal ear vein twice before inoculation with virus, and regularly thereafter with a 3-day interval between successive sampling. After separation, the sera were stored at −30°C and inactivated at 56°C for 30 min prior to testing. All rabbits were examined clinically each day. Measurement of body temperature was made per rectum.

Virus

Rabbits were injected with the Nakamura III strain of rabbit-adapted rinderpest virus (Nakamura, Wagatuma & Fukusho, 1938), which until recently was commonly used for the immunization of cattle against rinderpest infection. This strain, derived by passage over 600 times through rabbits after primary isolation from cattle, is highly virulent for certain breeds of rabbit, producing pathognomonic lesions in the lymphoid structures associated with the gastro-enteric tract. It was received from the Federal Department of Veterinary Research, Vom, Northern Nigeria in the form of freeze-dried splenic tissue of infected rabbits. This was reconstituted in ice-cold phosphate-buffered saline (PBS) (pH 7) and diluted in the same buffer to the required infective dose. The diluted material was kept on ice and shielded from direct sunlight until administered intravenously within 30 min of reconstitution.

Antigen

Chicken blood was obtained on the day of use by bleeding from the brachial vein into a tube containing sequestrene. The erythrocytes were washed three times by centrifugation and finally resuspended in PBS at 1% (v/v). One millilitre of this antigen was given intravenously 3 days after challenge with virus and in some instances a second dose was given approximately 3 weeks later.

Haemagglutinin test

Serial two-fold dilutions of inactivated serum were made in plastic trays using PBS in 0·1-ml volumes. An equal volume of 1% washed erythrocytes was added and the titre read macroscopically after 1 hr at room temperature.

RESULTS

The effect of viral concentration on the primary immune response to chicken erythrocytes is shown in Fig. 1. A marked depression was noted with certain levels of virus. This, however, did not appear to be related to viral concentration, all rabbits given virus above the minimal effective level showed a similar degree of depression regardless of dose.

It was also noted that immunodepression only occurred in those rabbits which became febrile following inoculation.

Repeat experiments confirmed these findings and enabled a standard dose of virus to be selected for subsequent studies which caused obvious immune depression and only caused

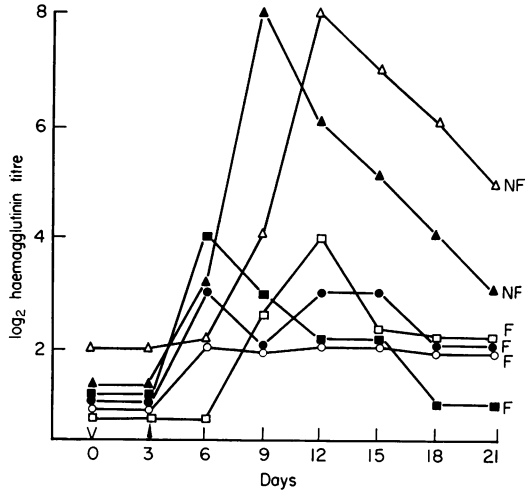

FIG. 1. The effect of virus at varying concentrations on the primary response to chicken erythrocytes. V, Inoculation of virus; ↟, injection of erythrocytes; ●, undiluted viral suspension; ○, 10^{-1} dilution of virus; ■, 10^{-2}; □, 10^{-3}; ▲, 10^{-4}; △, PBS only; F, febrile; NF, non-febrile.

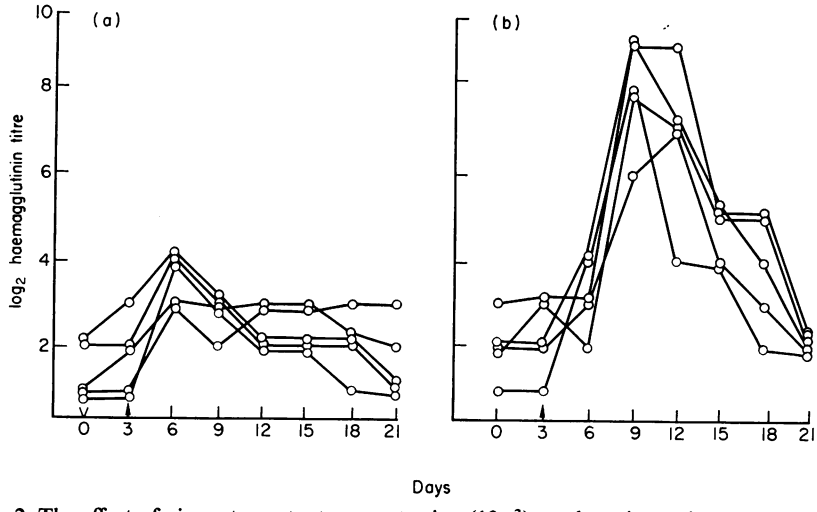

FIG. 2. The effect of virus at constant concentration (10^{-3}) on the primary immune response to chicken erythrocytes. (a) Virus inoculated rabbits, (b) normal rabbits. V, Inoculation of virus; ↟, injection of erythrocytes.

mild clinical signs which included pyrexia, dullness and diarrhoea and these were usually only observed for a 24–48-hr period commencing approximately 48 hr after inoculation. The effect of virus at this concentration on the primary response to chicken erythrocytes is

depicted in Fig. 2(a). All virus-infected rabbits failed to develop titres of antibody comparable to control animals (Fig. 2b). Depression was of the order of four to five serial two-fold dilution stages indicating that ten to twenty times less antibody was produced by these rabbits as compared to unchallenged control rabbits. Application of Student's *t*-test showed this degree of depression to be highly significant. Although maximum haemagglutinin titres were markedly reduced, neither the induction period nor the initial rate of antibody production were obviously altered by infection.

In subsequent studies the response of virus-inoculated rabbits which had shown depressed primary responses to a second antigenic stimulus given approximately 3 weeks after the primary inoculation was investigated. These rabbits received virus before the first antigen injection only. No effect on the secondary response was noted in three out of five rabbits under this treatment but the remaining rabbits showed impaired responses (Fig. 3a). In one case no haemagglutinin production was observed whilst in the other the titre reached only one quarter of that expected. In contrast, all normal rabbits produced typical secondary responses (Fig. 3b).

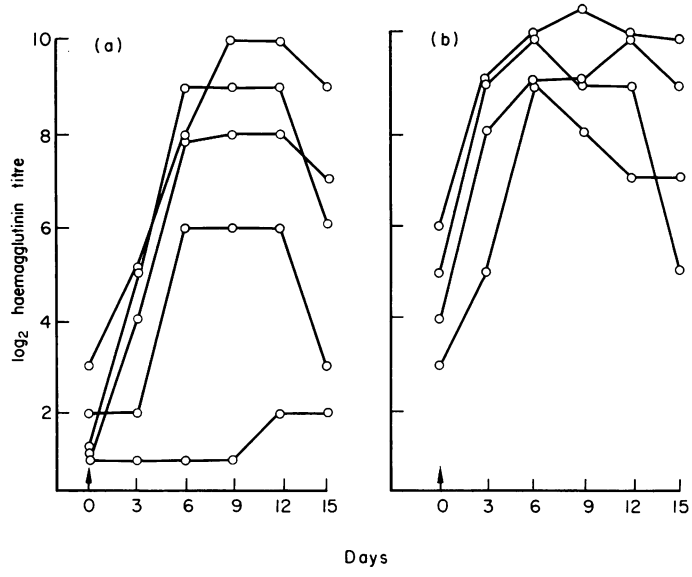

FIG. 3. The effect of virus given before initial antigen injection on the secondary response to erythrocytes given 3 weeks later. (a) Rabbits with depressed primary responses, (b) normal rabbits. ▲, Injection of erythrocytes.

DISCUSSION

In the present study it was found that even though haemagglutinin titres were markedly reduced, the induction period of the response was not increased nor was the initial rate of antibody production obviously affected. This suggests that the initial phases of the response are not influenced by the virus. A similar effect has been found with the oncogenic Friend virus (Salaman & Wedderburn, 1966). However, in this preliminary investigation the effect of challenge at varying intervals before antigenic stimulation was not investigated and it is

possible that if a longer interval had been selected the induction period and early antibody response may have also been affected.

It is conceivable that the reduced immune response to chicken erythrocytes following pretreatment with virus was due at least in part, to competition between viral and erythrocyte antigens. However, antigenic competition of this nature could not account for the effect on the secondary response which was observed in some cases where the erythrocyte and viral antigen injections were widely spaced.

Despite the very rapid clinical recovery from the effects of the virus, antibody production was not subsequently observed even though the rabbits appeared to be in good condition and antigen was still likely to be available at this time. It is possible that this may be accounted for by the development of tolerance following the initial inhibitory phase, as a result of which surviving or newly produced immunologically competent cells may be in a receptive condition for the induction of this state. In support of this possibility is the observation that two of the rabbits given a second antigenic stimulus did not exhibit typical secondary responses. Had the interval between the two injections of antigen been shorter it is possible that more rabbits may have failed to respond.

It was fortuitous that in the present studies the infectivity of the particular batch of virus used was clearly very low, presumably due to inactivation by adverse storage conditions or other factors. This enabled the effect of the virus to be investigated over the entire period of the immune response and contrasts with the experience of other workers with this strain where very high mortality rates were observed which were not dose-dependent (Scott, 1959). In consequence it is necessary to exercise caution in drawing general conclusions regarding the influence of viral concentration from the present limited experiments.

On the other hand it was considered particularly significant that immunodepression was only observed in febrile rabbits. This leads to the conclusion that rabbits in which infection, that is virus multiplication, was established were depressed immunologically whereas rabbits in which the virus failed to multiply were not affected. This suggests that immune depression was an important factor in the pathogenesis of the syndrome and it is possible that it precedes, and is the direct cause of the major clinical and pathological manifestations. Similarly, the lesions and symptoms in the natural disease in cattle are consistent with immunological failure. Immune depression may enable not only the virus to spread rapidly, unhampered by the usual immunological defence mechanisms, but may also encourage the uncontrolled proliferation of many of the normally commensal micro organisms as well. It is possible that the major pathological changes of rinderpest are due to this second factor rather than by direct action of virus.

In view of the very numerous studies of the immune responses to viruses it is surprising that records of virus immunodepression are rare. This may be either because it is indeed an uncommon phenomenon or because such effects have been overlooked. As far as the second possibility is concerned it is not unlikely that, although observed, immune depression may have been confused in some instances with poor antigenicity of the virus concerned. It is only when the effect of virus on the response to a standard antigen with well defined activity is examined that inhibition may become clearly evident. Studies of this nature have not been made until recently (Salaman & Wedderburn, 1966).

As the first indication of immune suppression by virus was found as early as 1908 by Von Pirquet, the scarcity of later records is even more surprising. Von Pirquet noted that the tuberculin reaction was suppressed in children with measles who had been previously

positive to this test. More recent observations of the effect of measles virus on tuberculin reactions have confirmed these early studies (Mellman & Wetton, 1963; Brody & McAlister, 1964). Influenza virus also exerts a suppressive effect upon the tuberculin reaction (Bloomfield & Mateer, 1919). It is probably highly significant that influenza, measles and rinderpest viruses are all at the present time classified together in the myxovirus group. It is likely, therefore, that immunosuppressive activity is a common feature of many, if not all viruses of this particular group and further evidence of immunosuppression should be sought amongst other members. Furthermore, the pathogenesis of the diseases caused by these viruses should also be reappraised in the light of this possible characteristic.

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