Evidence for a Selective Cytotoxic Effect of Carrageenan on Cells of the Immune System in vivo and in vitro

A. W. Thomson[1], A. R. Wilson, W. J. Cruickshank and Audrey H. Jeffries

Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN (Scotland), 11 November 1975.

Summary. Carrageenan suppressed antibody production to sheep erythrocytes in the mouse only when injected together with or 24 h prior to antigen. Pretreatment of sensitized spleen cell cultures with carrageenan reduced the degree of stimulation obtained using antigen but not PHA.

There is evidence from studies on cultured cells that the high molecular weight sulphated polygalactose carrageenan, derived from the marine alga *Chondrus crispus*, is toxic to macrophages but not cytotoxic to lymphocytes[2,3]. Such an agent could prove an asset in elucidating the role of the macrophage in immune reactivity. Recently it has been shown that carrageenan injection suppresses production of serum antibody and splenic plaque-forming cells (PFC) to sheep red blood cells (SRBC) in the mouse[4,5]. However, the stage(s) during the development of an immune response at which carrageenan exerts its immunosuppressive effect has not been ascertained.

In this study we have investigated, in the mouse, whether a relationship exists between time of carrageenan administration in relation to antigen (SRBC) and the ensuing level of humoral antibody production. Also, we have examined, in vitro, the effect of carrageenan on the response of sensitized spleen cells to both antigen and the lymphocyte-stimulating agent phytohaemagglutinin (PHA).

Materials and methods. Male LACA mice weighing 25–30 g were injected i.p. with 1×10^8 washed sheep red blood cells (SRBC) (Wellcome Reagents Ltd.) in 0.2 ml phosphate buffered saline (PBS) pH 7.2. Potassium carrageenan (Sigma Chemical Company) was dissolved either in warm PBS or, for addition to cell cultures, in warm culture medium (see below) then sterilized by membrane filtration. Animals received 0.5 mg i.p. at various times in relation to antigen. Control mice were injected with the same amount (0.2 ml) of PBS. 96 h after antigen injection mice were killed and direct (IgM) antibody plaque-forming cells (PFC) in the spleen estimated by the Cunningham technique, as described by Dresser and Greaves[6]. The number of PFC obtained per million spleen cells was expressed as percent control value.

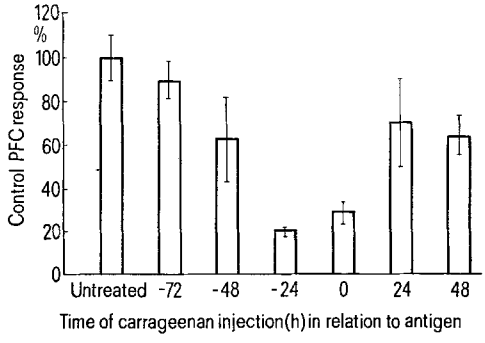

Fig. 1. Direct PFC responses 96 h after antigen administration in the spleens of male LACA mice receiving a single carrageenan injection at various times in relation to antigen. Columns represent mean values ± 1 standard error obtained from 10 animals.

The effect of carrageenan on cultured lymphoid cells was investigated using spleen cells from an LACA mouse injected one month earlier with 0.2 ml 5% SRBC. Replicate cultures were set up in capped $3'' \times 1/2''$ tubes and comprized 1×10^6 nucleated cells in 0.3 ml Eagle's minimum essential medium (MEM) (Biocult Laboratories) supplemented with 10% heat inactivated autologous serum. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 in air. Soluble antigen was prepared by first lysing SRBC in distilled water at a concentration of 1×10^7 cells/ml then diluting the preparation 2-fold in double-strength culture medium. Antigen solution (0.2 ml) was added to the appropriate tubes 6 h after establishment of the cultures. Carrageenan (150 µg) was added either at the start of the culture period or 6 h after introduction of antigen.

Additional cultures, to which either carrageenan or medium had been added were treated with 50 µg phytohaemagglutinin (Wellcome, PHA-P) at 6 h. At 48 h,

[1] Present address: Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen AB9 2ZD, Scotland.

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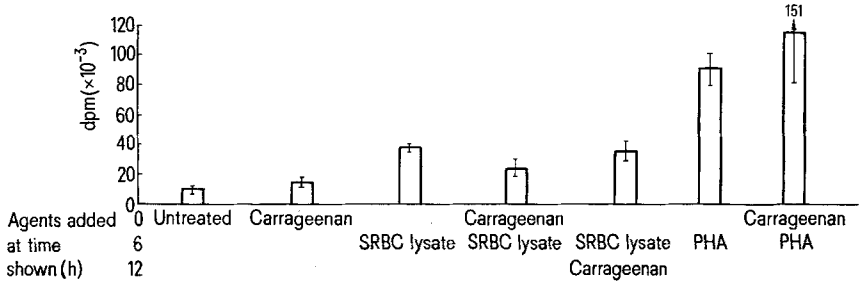

Fig. 2. Effect of carrageenan on antigen and PHA-stimulated tritiated thymidine incorporation by sensitized LACA mouse spleen cells. Columns represent mean values ± 1 standard error of 5 cultures.

2 μCi ^{3}H-thymidine (Radiochemicals Centre, Amersham) was added to each culture. 3 h later the cell deposit was washed consecutively in PBS, 5% trichloracetic acid (3 times), absolute methanol then dissolved in 0.2 ml 0.1 *M* NaOH. The ^{3}H content was determined by scintillation counting after mixing NE 260 scintillation fluid (Nuclear Enterprises Ltd.).

Results. Figure 1 shows that the level of PFC production 96 h after SRBC injection was dependent on the time of carrageenan administration in relation to antigen. Antibody production in mice receiving a single injection at −24 or 0 h were significantly lower than control values ($p < 0.005$ and $p < 0.0025$ respectively, according to Student *t*-test), the effect being most marked when carrageenan was given at −24 h. Carrageenan treatment at any other time did not significantly affect the response.

The effect of carrageenan on cultures of in vivo sensitized spleen cells is shown in Figure 2. Notably, the presence of carrageenan alone did not significantly affect the amount of thymidine incorporated. Although pretreatment of antigen-stimulated cells with carrageenan significantly depressed the response ($p < 0.025$), the addition of this agent 6 h after antigen challenge did not affect uptake of radiolabel. The level of stimulation obtained to the polyclonal mitogen PHA was not significantly altered by pretreatment of the spleen cells with carrageenan.

Discussion. From this study it is clear that the efficacy of carrageenan as an immunosuppressant both in vivo and in vitro depends on the temporal relationship between treatment and antigen administration. The fact that carrageenan caused marked suppression of the PFC response only when given 24 h before or together with SRBC suggests that it acts on the inductive phase of the immune response and not on antibody-producing bone-marrow derived (B) cells. Macrophages are known to be involved in the induction of immune reactivity to a variety of antigens[7] and our observations are therefore consistent with the known in vitro selective cytotoxic effect of carrageenan for these cells. This argument gains further support from our finding that treatment of sensitized spleen cell cultures with carrageenan before, but not 6 h after antigen challenge impaired the blastogenic response.

It is unlikely that carrageenan is toxic to thymus-derived (T) lymphocytes, since it failed to suppress lymphocyte transformation by PHA, a known T cell mitogen. This finding is in agreement with the observation of previous authors using cultured human and guinea-pig lymphocytes[2,8]. Further, we have shown elsewhere[9] that in the rat, carrageenan does not affect T cell function as measured by the graft versus host reaction. In conclusion, we believe that these observations are consistent with the hypothesis that in vivo, carrageenan is toxic to macrophages but non-toxic to lymphocytes.

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Production of Heteroagglutinins in Haemocytes of *Leucophaea maderae* L.

G. A. AMIRANTE[1]

Laboratorio di Zoologia dell'Università, Via Celoria 10, I-20133 Milano (Italy), 12 May 1975.

Summary. It was our purpose to study the immunological activity of circulating fluid cells in the blattoid insect *Leucophaea maderae* L. These cells are generically called haemocytes; after a preliminary morphological study under optical microscope, they were treated with anti-heteroagglutinins serum marked with fluorescein isothiocyanate. Thus, it was possible to show the presence of heteroagglutinins into the cytoplasm, as regards one group of haemocytes, and on the cell membrane for the second group.

For some years the attention of many researchers has been drawn more and more to the immunological phenomena to be found in invertebrates.

The substances present in the fluids of many invertebrate classes and in nearly all species, are the object of studies which more and more frequently appear in the literature. These substances are commonly known as heteroagglutinins, due to their capacity for reacting more or less specifically with heterologous cells or with bacteria.

Such substances have been isolated from worms[2,3], molluscs[4,5] arthropods[6–10], echinoderms[11,12], and protochordates[13,14], and many authors consider them to be similar to or comparable with vertebrate immunoglobulins. Some authors also suggest that the immunoglobulinic chains of vertebrates may originate from these simpler proteins. In this connection, the heteroagglutinins of some crustacean and mollusc species have been studied particularly from a structural, immunochemical and biophysical point of view[15].

On the other hand, nothing is known about organs, tissues or cells that may be involved with, or responsible for their synthesis, except for MARCK's studies[8] concerning

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