branaire existe dans le matériel nucléaire. Elle est décelable sur les contrastes négatifs (figure 2) de la rickettsie de type II chez laquelle elle est très développée (figure 6).
Les corps initiaux (figures 3 et 5). Les corps initiaux de la rickettsie de type I (figure 3) sont coccoïdes, d'environs 400 nm de diamètre; ceux de la rickettsie de type II (figure 5) sont bacilliformes, de 1200 pm de long sur 450 nm de large. Ils montrent cependant la même organisation: limités par 2 membranes unitaires séparées, ils ont un cytoplasme clair aux électrons au sein duquel sont visibles de nombreuses fibrilles denses ainsi que des granules ribosomiens.
Les corps intermédiaires (figures 3 et 5). Formes de transition entre les 2 formes précédentes, on y observe une densification latérale correspondant au regroupement du matériel nucléaire et une restructuration progressive des limites cellulaires. Lenticulaires chez la rickettsie de type I (figure 3), les corps intermédiaires sont bacilliformes chez la rickettsie de type II (figure 5).
Chez la rickettsie de type II, des corps géants sont visibles (figure 7). Parasphériques, ils atteignent 2,5 μm de diamètre et renferment des structures allongées constituées de fibres accolées. En section transversale, ces structures présentent une maille cristalline.

*Discussion.* Le cycle intracellulaire des 2 microorganismes permet de les placer dans l'ordre des Chlamydiales[5]. Leurs caractères ultrastructuraux, en particulier ceux des corps élémentaires, traduisent leur appartenance au genre *Porochlamydia*[7] qui va donc s'enrichir d'une ou de deux espèces nouvelles. Il n'est pas en effet certain que la rickettsie de type I soit une nouvelle espèce. Trouvée chez les araignées dans des stations très voisines de celles où les scorpions sont infectés par *Porochlamydia buthi*[7], elle ne présente, avec ce microorganisme, que de légères différences morphologiques au niveau des corps initiaux et des corps intermédiaires. Son élévation au rang d'espèce est donc subordonnée à la mise en évidence de caractères spécifiques, d'ordre immunologique par exemple, et aux résultats d'essais d'infections croisées montrant la spécificité d'hôte des 2 microorganismes. En ce qui concerne la rickettsie de type II, qui possède des caractères ultrastructuraux originaux, on peut en revanche la considérer dès à présent comme une nouvelle *Porochlamydia*. L'établissement de la diagnose de cette espèce nécessite un complément d'information sur ses affinités immunologiques ainsi que sur son cycle où l'on note la présence de corps géants, jusqu'alors inconnus dans l'ordre des Chlamydiales.

## Neutrophils are mediators of antiviral immunity[1]

B. T. Rouse, L. A. Babiuk and P. M. Henson

*Department of Veterinary Microbiology, University of Tennessee, Knoxville (Tennessee 37916, USA); Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon (Saskatchewan, Canada) and National Jewish Hospital, Denver (Colorado, USA), 4 July 1977*

*Summary.* The paper presents evidence that polymorphonuclear neutrophils upon stimulation with herpesvirus-induced antigens release a material inhibitory to virus infection. The material does not appear to be identical to type I or II interferon.

It has been customary to consider the polymorphonuclear neutrophil (PMN) as an effector cell primarily involved in antibacterial rather than in antiviral defense. Recovery from viral infections seems to be the domain of the macrophage, type I interferon produced by virus-infected cells and several components of specific immunity[2–6]. Amongst these latter specific components, antiviral cytotoxic T cells and the lymphokine type 2 interferon are assumed to be the most important effector mechanisms of recovery. Although PMN could play a role in antiviral defense by phagocytosing opsonized virus particles, the importance of this process in controlling virus dissemination in vivo has not been defined. Since PMN produce little or no interferon[7,8], they may not be involved in recovery from viral infections. However, some recent observations have suggested a part for PMN in antiviral immunity[9–12]. For instance, PMN were shown to act as effector cells in antibody-dependent cell cytotoxicity (ADCC), which in turn was assumed to provide an in vitro model of antiviral defense[6,13,14]. In fact, in the bovine species, PMN were the most efficient of all cell types tested at mediating antiviral ADCC[10]. Furthermore, PMN, in the presence of antibody, could prevent virus dissemination when added to virus-infected monolayers[9,15]. We now report further evidence that PMN may be involved in antiviral immunity by showing that the PMN's are induced, by exposure to herpesvirus antigens, to release subcellular mediators which can render cells resistant to virus infection.

Table 1. Stimulants examined which failed to cause bovine neutrophils to release virus inhibitor

| |
|---|
| Vesicular stomatitis virus infected GBK cells |
| Bovine rotavirus infected BSC-1 cells |
| Bovine virus diarrhoea infected GBK cells |
| Parainfluenza III virus infected GBK cells |
| Newcastle disease virus infected GBK cells |
| Herpes simplex virus infected VeRo cells |
| Canine distemper virus infected dog kidney cells |
| Heat aggregated bovine gamma globulin (500 μg/ml) |
| Purified protein derivative of human tuberculin (500 μg/ml) |
| Antibody and complement opsonized zymosan particles |

*Materials and methods.* Bovine PMN from animals with no known exposure to the viruses under investigation were isolated from the mammary glands by methods reported previously[16]. These cells consisted of 98–99% PMN and were contaminated by macrophages. The cells were suspended in Eagles' base minimal essential medium (MEM) containing 1% fetal calf serum at a cell concentration of $5 \times 10^6$/ml.
Preparation of cell and viral stimulants. The following cell lines were cultured in 100-mm petri dishes (approximately $8 \times 10^6$ cells) in MEM containing 5% FCS – Georgia bovine kidney cells (GBK), 2 monkey kidney cell lines – BSC-1 and VeRo and dog kidney cells. The viruses shown

Table 2. Biologic properties of various soluble mediators which interfere with virus replication

| Mediator* | Activity in different cells | | | Acid sensitivity (pH 2***) | Virus neutralization activity |
|---|---|---|---|---|---|
| | Porcine** | Equine | Bovine | | |
| Type I interferon | 40 ± 10 (12.5) | 60 ± 12 (19) | 320 ± 20 | 309 ± 15 (96) | None |
| Type II interferon | 2100 ± 20 (140) | 512 ± 14 (34) | 1500 ± 40 | 256 ± 30 (17) | None |
| PMN mediator (polyferon) | 560 ± 10 (98) | 640 ± 20 (114) | 580 ± 14 | 620 ± 18 (106) | None |

* All mediators are of bovine origin, type I interferon was produced by stimulation of bovine skin fibroblast cultures with Newcastle Disease virus at a multiplicity of infection of 0.5 for 24 h. Type II interferon was produced by stimulating bovine T lymphocyte-macrophage cultures with UV-irradiated IBR virus as described previously[5]. In all instances, the material was centrifuged for 3 h at 100,000 × g to remove residual virus. ** A continuous porcine kidney cell line (ESK) and a continuous equine dermis (E-derm) cell line obtained from American Type Culture Collection. Numbers in brackets represent the percentage of the activity demonstrated in homologous bovine cells. *** Mediators were treated at pH-2 for 24 h at 4°C neutralized and titrated on bovine cell cultures. Controls were incubated at 4°C in parallel.

in table 1 were cultured. In addition, GBK cells were infected with infectious bovine rhinotracheitis virus (IBR) also at a MOI of 1. Cells were harvested when showing advanced signs of CPE. To effect this, the fluids were removed, the monolayers washed twice in Hank's balanced salt solution (HBSS) and the infected cells UV-irradiated to inactivate virus. Uninfected control cells were treated likewise. After irradiation, the cells were detached from the plastic with a rubber policemen, resuspended to 2 ml in HBSS and stored at −70°C. Prior to use, the antigen was thawed, agitated and diluted as required and added in 50 µl aliquots to 1 ml cultures of PMN in 1.6 mm wells of 4 × 6 Linbro culture plates. The concentration of stimulating material was expressed in units with 1 unit being equivalent to $4 \times 10^3$ cells (infected or control) prior to storage at −70°C. The cultures were incubated at 37°C for 24 h (in all experiments except the kinetics of release experiments), after which the supernatant fluids were harvested, briefly centrifuged to remove cells and stored at −20°C until assayed for inhibitor activity.

Cell free IBR virus was also used as a stimulant. This was obtained by collecting the fluids from IBR-infected GBK cells (at 24 h). To effect concentration, these fluids were centrifuged at 100,000 × g for 3 h, the pellet resuspended to 1/50th the original volume in HBSS and UV-irradiated to inactivate infectivity. Heat-aggregated bovine ganna globulin (BGG) was prepared by heating commercial BGG (Sigma) at 63°C for 30 min. Antibody and complement opsonized zymosan particles were prepared exactly as described by Henson[17].

Titration of antiviral inhibitors. The method used was as described previously[5]. Briefly, 2 fold dilutions of supernatnats collected from the stimulated or unstimulated PMN cultures, were added in 100-µl volumes to monolayers of GBK cells in microtiter plates. 24 h later, the fluids were removed, the monolayers washed, and infected with either 25 plaque forming units of vescicular stomatitis virus (VSV) or IBR virus. 1 h later, the unabsorbed virus was removed and the VSV-infected cultures were overlayed with 1% methyl cellulose in MEM. In the case of IBR-infected cultures, the fluids to be titrated were added back (in 100 µl volumes) together with 100 µl MEM + 10% FCS containing 4 neutralizing units of anti-IBR serum[5]. In some of the control monolayers, the stimulants were added to MEM at the concentration used to stimulate PMN. This was to control for any interferon producing activity of the remaining antigen in the preparation being titrated for antiviral activity.

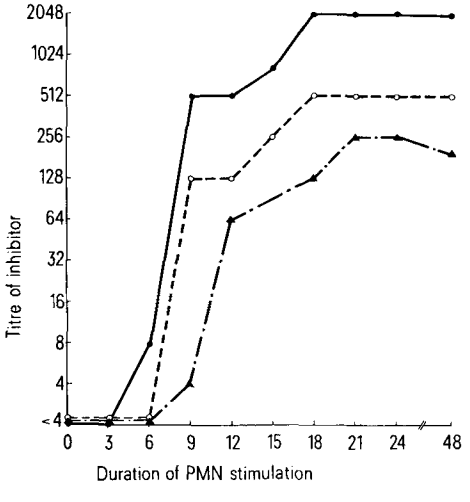


Fig. 1. Kinetics of inhibitor production following stimulation of neutrophils with IBR-virus-infected Georgia Bovine Kidney cells. The results of 3 experiments are shown. Uninfected GBK cells at the same concentration failed to stimulate inhibitor release. Sonicates of bovine PMN also expressed no virus-neutralizing activity or were able to protect GBK cells against VSV or IBR virus replication.

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In all cultures, after 48 h, the monolayers were washed and stained and the plaque inhibition titer recorded. The PMN supernatants were also examined for virus neutralizing activity against VSV and IBR by methods described before[5]; no neutralizing activity was found.

The details of the methodology used to produce bovine types I and II interferons were described previously[18]. Also described previously were the methods of measuring acid sensitivity and the antiviral activity of the compounds in different cell lines[18].

*Results and discussion.* As is shown in figure 1, IBR-virus-infected GBK cells (IBR-GBK) was a potent stimulator for the release of an antiviral inhibitior from the bovine PMN cultures. Thus the cell free supernatant fluid, from such cultures had antiviral activity against both VSV and IBR viruses. The stimulatory effect of the IBR-GBK antigen was dose dependent and was apparently the result of stimulation of PMN by IBR viral induced antigen since uninfected GBK cells failed to cause the release of any inhibitor. As few as approximately 1000 virus-infected cells were needed to cause the PMN cultures to release detectable inhibitor.

The inhibitor was first detectable 6–9 h after stimulation, reached peak levels at 18–21 h and persisted for at least 48 h (figure 2). The activity was expressed against both VSV and IBR viruses. Although virus-infected cells caused the release of large amounts of inhibitor, the levels of free virus released from such cells were insufficient to stimulate release. In fact it was necessary to concentrate virus 10fold or more for it to be stimulatory (equivalent to virus released from approximately $4 \times 10^5$ infected GBK cells). Since such cell-free virus could additionally contain cell membrane fragments, it is possible that such fragments, with incorporated virus-coded antigen, were responsible for the stimulation.

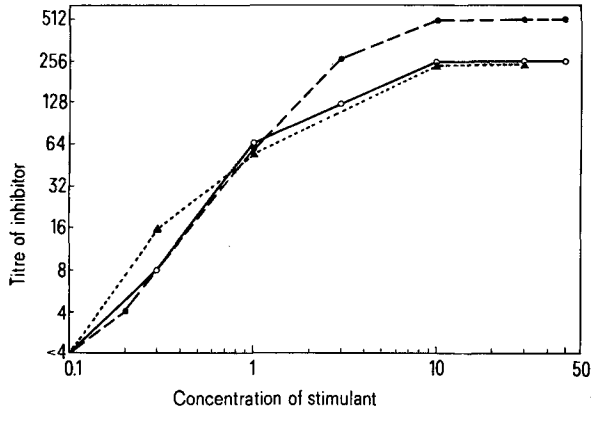


Fig. 2. Kinetics of production of inhibitor of VSV virus replication after stimulation of PMN with UV-irradiated IBR-virus-infected GBK cells. The results of 3 separate experiments are shown.

Whereas GBK cells infected with IBR virus regularly triggered PMN to release the inhibitor, other virus-infected cells tested were unable to do so (table 1). These observations may mean that the PMN bear a receptor that specifically recognizes a herpesvirus. However, many more viruses must be investigated and direct binding studies must be done, before such a claim can be substantiated.

It is of interest that our observation may not merely be restricted to the bovine system, since we have observed, in preliminary experiments, that human PMN can be triggered by Herpes simplex virus infected VeRo cells to release a similar inhibitor (unpublished data). Although the antigen recognition event required for the induction of the inhibitor may prove to be virus-specific, the material itself is, like type I interferon, nonspecific in that the activity was expressed against both DNA and RNA viruses. Also in resemblance ot interferon, the material exhibited no direct antiviral effects as could be determined by virus neutralization assays. However, preliminary characterization studies have revealed differences from bovine type I and type II interferons, in that the material shows an intermediate level of acid sensitivity and antiviral activity in some heterologous cells (table 2).

Studies are in progress not only to characterize the inhibitory material, but also to describe the optimal conditions needed for its production. It seems not to be performed, since sonicates of PMN were not inhibitory (table 2). The contents of PMN granules did not appear to be the inhibitor since treatment of PMN with antibody-complement opsonized zymosan particles, a procedure that causes the release of granule contents[17], failed to trigger the release of the inhibitor (table 1).

Whatever the nature of the inhibitor or the exact conditions needed for its production, our studies do serve to strengthen the hypothesis that PMN may be extremely important effector cells in antiviral immunity. It might also be that we have identified a 3rd, type of interferon – if so we could provisionally name the material interferon 3.

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## Foliar spirality and aestivation of flowers in *Hibiscus cannabinus* Linn.

S. S. Ghosh and T. A. Davis[1]

*Crop Science Unit, Indian Statistical Institute, Calcutta 700 035 (India), 5 July 1977*

*Summary.* In *Hibiscus cannabinus* a negative association exists between the foliar spirality and the aestivation of corolla. Moreover, it is seen that the fruits developed from left-twisting flowers of left-spiralled plants and those of the right-twisting flowers of right-spiralled plants are better in quality.

The aestivation of corolla in *Hibiscus cannabinus* is contorted (the 5 petals twist regularly) as in other species of Malvaceae[2–4]. In about 50% of the flowers on any of these annual plants, the contortion of petals is clockwise (left-handed) and in the rest, counter-clockwise (right-handed). Since the phyllotaxy in this species is alternate (cyclic), the plant/shoot can be grouped into left-handed or right-handed[5]. The foliar asymmetry is not genetically determined. However, there is an association between the foliar spirality and the aestivation of petals. A left-handed shoot is found to produce more of right-twisting (counter-clockwise) flowers, and this situation is reversed in the case of