Effect of Recombinant DNA-produced Bovine Interferon Alpha (BoIFN-α₁) on the Interaction between Bovine Alveolar Macrophages and Bovine Herpesvirus Type 1

By H. BIELEFELDT OHMANN,* J. E. GILCHRIST AND L. A. BABIUK
Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, and Veterinary Infectious Disease Organization, Saskatoon, Saskatchewan, Canada, S7N 0WO

(Accepted 4 June 1984)

SUMMARY

Treatment of bovine alveolar macrophages (BAM) with bovine leukocyte interferon (BoIFN-α₁) resulted in reduced bovine herpesvirus type 1 replication and spread. This was demonstrated by reduced virus yields and number of cells infected. BoIFN-α₁ treatment of BAM also induced enhanced Fc receptor expression and/or avidity by the cells, increased their activity in antibody-dependent cellular cytotoxicity, and augmented their extrinsic antiviral activity as measured by a reduction in the development of plaques in a susceptible cell line. These results are discussed in the context of the possible role of interferon in activation of AM during the early phases of a virus infection.

INTRODUCTION

The mononuclear phagocyte system constitutes a central component of the antimicrobial defence system of an animal, expressing both non-specific and immune-specific functions (Van Furth, 1980). In the lung, the resident alveolar macrophages (AM) and monocytes, migrating into the lungs from the blood, offer the first line of cellular defence against viral and bacterial invasion. Moreover, by interacting with other components of the immune system, the AM can regulate the antigen-specific immune response, such as antibody production and induction of cytotoxic T lymphocytes (Lyons & Lipscomb, 1983). The immediate tissue reaction to virus invasion is the production of interferon (IFN), which has been shown to modulate various macrophage activities both in vivo and in vitro (for review, see deMaeyer & DeMaeyer-Guignard, 1982). In this context the enhanced cytotoxic (Stanwick et al., 1980, 1982) and antibacterial (Bielefeldt Ohmann & Babiuk, 1984) activities of macrophages are of interest, as lung infections by viruses are often complicated by secondary bacterial invasion (Loosli, 1973), and it has been suspected that this is due to an impairment of AM functions (for review, see Forman et al., 1982a).

The interaction between bovine AM (BAM) and bovine herpesvirus type 1 (BHV-1) presents an interesting model for studying AM–virus interaction and determining why discrepancies occur between observations in vivo and in vitro (Nugent & Pesanti, 1979; Warshauer et al., 1977). BAM are susceptible to infection with BHV-1 in vitro, resulting in cytopathology and impairment of various effector functions (Forman & Babiuk, 1982; Forman et al., 1982b), but less than 0·1 % of lavaged cells from experimentally infected calves are productively infected with BHV-1 and BAM activities are unaltered (Forman et al., 1982a). These results indicate that factors in the lung microenvironment may render the BAM resistant to BHV-1 infection. One such factor could be IFN(s) (Rodgers & Mims, 1982). To date, it has not been possible to determine unequivocally whether IFN or some other factor is responsible for these effects. With the advent of recombinant DNA technology for production of IFNs, it has now become possible to use homogeneous preparations of bovine IFN to demonstrate unambiguously the influence of IFN on virus susceptibility of BAM. In the present communication we report on the in vitro effects of recombinant bovine alpha interferon (BoIFN-α₁) on intrinsic and extrinsic antiviral activities (Morahan & Morse, 1979) of BAM as expressed against BHV-1.
METHODS

Calves. Holstein and Holstein-Friesian calves were obtained by Cesarian sections and artificially reared in isolation. Two age groups were employed: calves 10 days to 2 months old and calves 6 to 12 months old. All calves remained negative for BHV-1 throughout the investigations as judged by periodic serological surveys.

Cells. Georgia bovine kidney (GBK) cells were cultured in Eagle’s base MEM supplemented with 10% heat-inactivated foetal bovine serum (FBS), non-essential amino acids, gentamicin and HEPES buffer as described previously (Rouse & Babiuk, 1974).

Alveolar macrophages were obtained by fibre optic bronchoscopic lavage, as described elsewhere (McGuire & Babiuk, 1982). After two washings in Hanks’ balanced salt solution (HBSS), cells were suspended in RPMI 1640 medium with 10% horse serum (HS), polymyxin B, penicillin and streptomycin, and either seeded in tissue culture plates as detailed below or incubated in suspension in polyethylene tubes with snap caps (no. 14-956-IF, Fisher Scientific, Edmonton, Alberta, Canada), under rotation at 2 to 4 r.p.m. at 37°C. Plated cultures were incubated for 2 to 3 h at 37°C in a humidified 5% CO2 atmosphere, washed twice with MEM and re-fed with RPMI 1640 containing 10% HS or FBS. Some cultures were treated with BoIFN-α, as detailed below.

Viruses and antisera. The origin and growth of BHV-1 strain P8-2 has been described previously (Rouse & Babiuk, 1974). Anti-BHV-1 serum was obtained as described by Babiuk & Rouse (1978).

Interferon. BoIFN-α, produced in Escherichia coli and purified to homogeneity (lot no. 1572-22a), was generously provided by Genentech Incorporated (South San Francisco, Ca., U.S.A.). The IFN preparation, which was greater than 99% pure, had a specific activity of 1.7 × 107 units/mg protein when titrated on Madin–Darby bovine kidney (MDBK) cells challenged with vesicular stomatitis virus (VSV) in a virus c.p.e. inhibition assay.

Viral replication in BAM. Alveolar macrophages were seeded in 24-well plates (Corning), at 1.5 × 106 to 2 × 106 cells/well. After 3 h of adhesion, cultures were rinsed and reincubated for 24 h with MEM/10% FBS with or without BoIFN-α in doses varying from 10 to 100 units/ml. Cultures were then rinsed thoroughly with MEM, inoculated with BHV-1 at an m.o.i. of 0.01, and incubated for 1 h at 37°C. Following two washings in MEM to remove unadsorbed virus, the cultures were reincubated with MEM/5% FBS. Subsequent procedures for harvesting of cultures and titration of infectivity by plaque assay on GBK cells were performed as described elsewhere (Forman et al., 1982b).

Immunofluorescence assay. Alveolar macrophages were seeded in eight-chamber slides (Lab-Tek Products, Westmont, Ill., U.S.A.), treated with BoIFN-α, as above, at doses ranging from 102 to 106 units/ml, followed by infection with BHV-1 in the usual manner. After 48 h of infection, the cultures were fixed and stained as described by Forman et al. (1982b).

Infectious centre assay. The experimental procedure followed a protocol previously described (Forman et al., 1982b). Briefly, BAM were cultured in 16mm diam. wells of cluster plates (Costar no. 3524), treated with BoIFN-α, for 24 h and infected with BHV-1 at an m.o.i. of 0.01. After adsorption for 1 h and removal of unadsorbed virus, the cells were reincubated with MEM/10% FBS containing 4 units anti-BHV-1 antibody. At various times post-infection the cells were removed from the wells by detachment with 9 mM-lidocaine solution (Astra Chemicals Ltd., Mississauga, Ont., Canada) in HBSS. The cell samples were washed, counted and diluted in MEM containing anti-BHV-1 serum. Triplicate fivefold dilutions of these cells were added in 1 ml volumes to confluent GBK cell monolayers in 24-well plates. After 48 h of incubation, the monolayers were fixed and stained, and virus plaques were enumerated microscopically.

Assay for interferon production by AM. AM were seeded in 24-well plates, treated with BoIFN-α, and infected with BHV-1 as described above. Culture fluids were harvested 24, 48 and 72 h after infection and centrifuged (1000 g) to remove cellular debris. After neutralization of BHV-1 in the supernatants by incubation with anti-BHV-1 serum for 1 h, levels of IFN activity were determined by the ability to inhibit VSV replication in GBK cells as described previously (Babiuk & Rouse, 1976).

Virus plaque inhibition assay. Virus plaque inhibition by BoIFN-α-treated AM was performed as described by Rouse & Babiuk (1975). AM were pretreated with BoIFN-α for 8 or 24 to 36 h prior to use as effector cells.

Assay for spontaneous cytototoxicity (SCC). AM (effector cells) were pretreated with BoIFN-α, for 24 to 36 h. GBK cells were grown in 25 cm2 culture flasks (Corning) in MEM with 10% FBS. When confluent, the monolayers were inoculated with BHV-1 at an m.o.i. of 1. After adsorption and removal of unadsorbed virus, the cultures were reincubated for 14 to 16 h with MEM/5% FBS. Cells were then dispersed by mild trypsinization, washed once and suspended at a concentration of 5 × 106 cells/ml in MEM/2% FBS and labelled for 1 h with 100 μCi Na251CrO4 per ml (New England Nuclear). Labelled target cells were washed three times with MEM/5% FBS and suspended at a final concentration of 5 × 104 cells/ml in the same medium. Mock-infected GBK cells were similarly prepared. The microcytotoxicity assay was performed according to Stanwick et al. (1980), with final effector to target cell ratios of 25 : 1, 50 : 1 and 100 : 1. Chromium release was calculated according to the formula: %51Cr release for SCC = 100 (2A/(A + B)), where A is the c.p.m. in 100 μl supernatant and B is the remaining radioactivity in the well (supernatant plus cell pellet), determined after lysis of the cells. Specific release was defined as
the $^{51}$Cr released from target cells in the presence of BAM minus the percentage released from target cells alone. The average $^{51}$Cr leakage from uninfected and infected cells was less than 30% in 24 h.

**Antibody-dependent cellular cytotoxicity (ADCC) assay.** The assay was performed as described previously (Rouse et al., 1976) with minor modifications. Target cells were prepared as described above for the SCT assay. BAM effector cells were incubated in the presence or absence of BoIFN-$
\alpha$ in polyethylene tubes (Fisher Scientific) in RPMI/10% HS at a cell concentration of $1.5 \times 10^6$ to $2.0 \times 10^6$ cells/ml under continuous rotation at 2 to 4 r.p.m., at 37°C for 3 to 4 h. Only cells isolated composed of more than 95% AM and monocytes as monitored by staining for non-specific esterase and/or differential counts on cytosmears stained with Wright's stain were used. Cells were washed three times in HBSS and suspended at various concentrations in MEM/5% FBS. The cytotoxicity assay was performed in round-bottom well microtitre plates at effector cell: target cell ratios of 50:1 and 25:1, and a final concentration of anti-BHV-1 serum of 1:50. The incubation was terminated after 6 or 15 h. 50% of the culture fluids aspirated and the radioactivity measured. Total releasable radioactivity was estimated as the amount released from target cells lysed in 5% Triton X-100. Controls included cultures containing either antibody-sensitized target cells alone or non-sensitized target cells plus BAM. Background release in all experiments was less than 10%. The specific release was estimated as: \% SR = (c.p.m. test - c.p.m. control)/(total releasable c.p.m. - c.p.m. control) x 100.

**Fc receptor activities.** Washed bovine red blood cells (BRBC), in a 5% suspension, were sensitized for 30 min at 37°C with rabbit anti-BRBC serum, washed and resuspended to a final concentration of 0.2% in MEM. BAM, pretreated with BoIFN-$\alpha$, as described for the ADCC assay, were dispensed in 200 μl volumes in Eppendorf tubes, pelleted, the medium was discarded and the cells were resuspended in 200 μl of sensitized BRBC. The mixture was then centrifuged at 40 g for 3 min and incubated for 30 min at 37°C. After incubation, the pellet was gently disrupted, a drop diluted in 0.1% trypan blue and examined by light microscopy. BAM binding three or more BRBC were considered to be Fc receptor (FcR)-positive. For estimation of FcR-mediated phagocytosis the BAM-BRBC mixture was again pelleted, resuspended in HBSS, diluted 1:4 with water to lyse attached BRBC, and reconstituted with double-strength MEM. A drop was again diluted in trypan blue and the number of cells containing one or more BRBC were scored. On a few occasions, the FcR activities were quantified by the use of chromium-labelled sensitized BRBC, as described elsewhere (Grewal et al., 1978).

**RESULTS**

**Effect of BoIFN-$\alpha$ on BHV-1 replication in BAM and endogenous IFN production**

Although virus replication is restricted in BAM as compared to bovine kidney cells, these cells do support virus growth (Forman et al., 1982b). Pretreatment of the BAM with BoIFN-$\alpha$ for 24 h before virus infection conferred partial protection, as revealed by both a reduction in the number of productively infected cells (Table 1), in virus yield and a delay in detectable virus production (Table 2). In accordance with the results of the virus titrations, a decrease in the size of the infected foci was noted both in the infectious centre assay and immunofluorescence assay. Analysis by PAGE of BoIFN-$\alpha$-pretreated and non-treated, BHV-1-infected BAM did not reveal any selective reduction in any of the virus-induced polypeptides (data not shown). In all assays designed to look at virus replication and spread, it was consistently observed that the optimum dose of BoIFN-$\alpha$ ranged between $10^2$ and $10^3$ units/ml. Higher quantities of BoIFN-$\alpha$, although still capable of reducing virus replication and spread, were less efficient. Thus, numbers of infected cells (Table 1) as well as virus yields (Table 2) were all larger than at lower

<table>
<thead>
<tr>
<th>Assay</th>
<th>Interferon treatment level (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>No. of fluorescing foci/well†</td>
<td>97$^\dagger$</td>
</tr>
<tr>
<td>Infectious centres$\S$</td>
<td>53125</td>
</tr>
</tbody>
</table>

$^\dagger$ Treatment was for 24 h prior to infection with BHV-1, strain P8-2. The results shown are representative of four or five separate experiments with each assay, performed with different animals.

$^\ddagger$ Infection dose was 100 p.f.u./well.

$^\S$ Number of two wells per IFN dose.

$^\S$ Number of infectious centres 56 h after infection of BAM.
Table 2. Effect of BoIFN-α₁ on BHV-1 replication in bovine alveolar macrophages

<table>
<thead>
<tr>
<th>Time of harvest (h)</th>
<th>Interferon treatment level (units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>70†</td>
</tr>
<tr>
<td>48</td>
<td>1.7 × 10³</td>
</tr>
<tr>
<td>72</td>
<td>6.5 × 10⁴</td>
</tr>
<tr>
<td>96</td>
<td>1.2 × 10⁴</td>
</tr>
</tbody>
</table>

* Treatment was for 24 h prior to infection with an m.o.i. of 0.01 of BHV-1 strain P8-2.
† Virus titre (p.f.u./ml) in culture supernatants, titrated on GBK cell monolayers.

Table 3. Effect of BoIFN-α₁ pretreatment of bovine alveolar macrophages on their production of interferon upon infection with BHV-1

<table>
<thead>
<tr>
<th>Calf no.</th>
<th>Interferon pretreatment dose (units/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>83–51</td>
<td>2048†</td>
</tr>
<tr>
<td>83–52</td>
<td>512</td>
</tr>
</tbody>
</table>

* Alveolar macrophages were pretreated for 24 h, washed twice and then infected with BHV-1 at an m.o.i. of 0.01.
† Interferon titre/ml in culture fluids determined at 48 h by inhibition of VSV replication on GBK cells after neutralization of BHV-1 with hyperimmune serum.

IFN concentrations. In addition to the antiviral effect of BoIFN-α₁ on BAM, an alteration of cellular metabolism was also detected. Thus, pretreatment of BAM with BoIFN-α₁ reduced the endogenous IFN production in BAM cultures, following BHV-1 infection, in a dose-dependent manner (Table 3).

Effects of BoIFN-α₁ on antibody-independent extrinsic antiviral activities of BAM

One of the modes by which some macrophage populations can express antiviral activity is revealed in vitro as a non-cytotoxic viral plaque inhibition (VPI) (Hayashi et al., 1980; Morahan et al., 1980; Rouse & Babiuk, 1975). As shown in Table 4, BAM also possess the capacity to inhibit viral plaque development in vitro, and this activity can be enhanced in a dose-dependent manner by pretreatment of the BAM with BoIFN-α₁ for 8 h, with maximal inhibition reached at the highest dose tested. The increase in VPI activity was reflected in an effect on plaque numbers as well as on size, without a general trend for either one or the other being dominant. The possibility that the observed effect was due to carry-over of IFN seems remote, as the procedure to procure the AM (non-treated and IFN-treated) by lidocaine detachment involved at least five or six washing sequences. Furthermore, if carry-over had been a problem, there should have been higher levels of IFN in the BAM treated with 10⁴ units/ml in Table 3, rather than lower levels as observed.

When BAM were pretreated for longer periods of time, i.e. 24 to 36 h, prior to employment in the VPI assay, the activity was either unchanged or even suppressed as compared to non-treated cells. The latter event was manifested as increased cytopathology in the BHV-1-infected GBK monolayer (data not shown). An attempt was therefore made to determine whether this apparent decrease in VPI activity represented a true inhibition of the BAM function or whether the prolonged IFN treatment had rendered the BAM cytotoxic to infected GBK cells, thus generating plaques that were not virus-induced. BAM, treated with BoIFN-α₁ for various times, were used as effector cells in a ⁵¹Cr release assay. In no case could we demonstrate spontaneous cell-mediated lysis of either infected or uninfected target cells (data not shown).

One of the mechanisms whereby BAM might exert the non-cytotoxic antiviral activity is by altering target cell metabolism (Hayashi et al., 1980; Keller, 1976; Morse & Morahan, 1981).
Table 4. Effect of BoIFN-α₁ pretreatment of bovine alveolar macrophages on their intrinsic antiviral activity as revealed by virus plaque inhibition

<table>
<thead>
<tr>
<th>Virus* (p.f.u./well)</th>
<th>Level of IFN pretreatment‡ (units/ml)</th>
<th>Virus control†</th>
<th>BAM added per well (× 10⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1.5 × 10²</td>
<td>1.5 × 10⁴</td>
</tr>
<tr>
<td></td>
<td>5-0</td>
<td>2-5</td>
<td>1-25</td>
</tr>
<tr>
<td>100</td>
<td>499-5</td>
<td>23-31</td>
<td>19-6</td>
</tr>
<tr>
<td>50</td>
<td>358-6</td>
<td>45-8</td>
<td>70-2</td>
</tr>
<tr>
<td>25</td>
<td>184</td>
<td>37-2</td>
<td>62-5</td>
</tr>
</tbody>
</table>

* Initial input virus/culture.
† Area of plaque formation in cultures without BAM added.
‡ BAM were treated for 8 h with various levels of BoIFN-α₁, washed and added to GBK cell cultures immediately following virus adsorption.
§ Number of plaques (N) × area of plaque (A).
‖ Percentage of plaque inhibition exhibited by BAM.

Table 5. Cytostasis mediated by bovine alveolar macrophages against GBK cells*

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Incorporation of [³H]thymidine†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. p.m. ± s. e.m.</td>
</tr>
<tr>
<td>Targets‡</td>
<td>2879 ± 214</td>
</tr>
<tr>
<td>Non-treated, 6 h</td>
<td>84-5</td>
</tr>
<tr>
<td>3.0 × 10² units/ml, 6 h</td>
<td>57-2</td>
</tr>
<tr>
<td>3.0 × 10⁵ units/ml, 6 h</td>
<td>46-4</td>
</tr>
<tr>
<td>Non-treated, 28 h</td>
<td>49-5</td>
</tr>
<tr>
<td>3.0 × 10² units/ml, 28 h</td>
<td>44-3</td>
</tr>
<tr>
<td>3.0 × 10⁵ units/ml, 28 h</td>
<td>41-8</td>
</tr>
</tbody>
</table>

* Target cells were cultivated for 12 h with or without BAM, either non-treated or pretreated with BoIFN-α₁, as indicated in MEM with 5% FBS. Labelling was for 1 h with 0.5 μCi/well of [³H]TdR.
† Percentage of incorporation as compared to GBK cells alone, was calculated after subtraction of [³H]TdR incorporation into BAM without target cells (300 c.p.m.), or BAM cultivated in the presence of mitomycin-treated GBK cells.
‡ GBK cells seeded at 1 × 10⁴ cells/well 12 h prior to addition of BAM.

Analysis of DNA synthesis in non-infected GBK cells co-cultivated with non-treated BAM revealed that BAM can reduce [³H]thymidine incorporation in the GBK cells in a dose-dependent manner. Similar results were obtained when [³H]cytidine was used (data not shown). BAM, cultivated alone or co-cultured with mitomycin-treated GBK cells, incorporate only negligible amounts of thymidine under the culture conditions employed [low serum content in medium (McGuire & Babiuk, 1982)]. Pretreatment of BAM with BoIFN-α₁ for 6 h substantially enhanced their cytostatic activity (Table 5). In contrast, the effect was only marginally apparent after 28 h of pretreatment when compared to the normally increased activity of control BAM caused by in vitro maturation and/or activation of the cells (Table 5). The arguments against possible carry-over of IFN, mentioned above, also apply to this experimental series.

Effect of BoIFN-α₁ on BAM ADCC and Fc receptor activities

Alveolar macrophages from young calves (2 months) express ADCC of BHV-1-infected cells rather slowly. However, pretreatment of the cells with BoIFN-α₁ boosted this activity. The effect was dose-dependent with maximal effect seen after 3 h at an IFN level of 1 × 10² units/ml (Fig. 1a). Incubation for longer periods (i.e. 6 h) did not result in a quantitative or qualitative change in activity. Results of experiments in which the expression of FcR activities were studied by rosette formation and phagocytosis of Ig-sensitized erythrocytes suggested that the enhanced ADCC activity was not only due to increased FcR expression and/or avidity (Fig. 1b, c, d), but
Fig. 1. Effect of pretreatment with BoIFN-α1 on expression of ADCC and Fc receptor activities by bovine alveolar macrophages. Plastic adherent (ADCC) or suspended (Fc receptor assays) AM were treated with various concentrations of BoIFN-α1 for 3 h, washed thoroughly and employed in the assays. (a) ADCC against BHV-1-infected cells. The results represent average activity of six independent experiments conducted with AM from different animals, expressed as percent of non-treated cells. [Specific release in 6 h assay was 13.2 (± 4.3)% Background release for target cells alone, for target cells and BAM without serum and for target cells and serum without BAM were less than 5%.] Bars indicate S.E.M. (b) Fc receptor-mediated rosette formation with sensitized (serum dilutions 1/100) erythrocytes. The results represent average of five separate experiments. Cells binding three or more erythrocytes represented 53.7 (± 8.3)% of the non-treated AM population. (c) Fc receptor-mediated phagocytosis. Cells binding one or more sensitized erythrocytes were counted and amounted to an average of 39.2 (± 15.8)% in five independent experiments. The results are given as percent of the activity in non-treated cells. (d) Quantification of FcR-mediated phagocytosis. BRBC were sensitized with 1/50 dilution of rabbit-anti-BRBC serum and labelled with 51Cr. The results are representative of three separate experiments done in triplicate on three different animals.

also the subsequent cytolytic mechanism(s) was increased. However, treatment with BoIFN-α1 did not increase the maximal ADCC activity, reached at 14 to 16 h of incubation above the normal plateau activity of control cells (results not shown), indicating that the effect of BoIFN-α1 is on the mechanism of ADCC rather than on the cytotoxicity.

DISCUSSION

In the study reported here it was demonstrated that pretreatment of BAM with BoIFN-α1 in vitro resulted in increased resistance to infection with BHV-1 and increased extrinsic antiviral activity of the cells as expressed by inhibition of spread of BHV-1 in bovine kidney cell cultures, and by ADCC. Thus, the results would appear to support the intriguing suggestion by Blanden et al. (1976), that the primary function of IFNs in recovery from viral infection might be to protect macrophages from those viruses capable of infecting them, and the cells then in turn can act as the principal mediators of specific and non-specific recovery processes.

Quite unexpectedly, it was found that the antiviral effect of BoIFN-α1 exerted on BHV-1
replication in BAM reached an optimum and then declined, resulting in a suboptimal effect at high levels of BoIFN-α, (Table 2). It is conceivable that the protective effect seen is the result of the combined activities of BoIFN-α and endogenous IFN produced by the BAM, the latter having a greater effect on the BAM than what is recognized from titration with VSV and GBK cells (Bell et al., 1983; Haller et al., 1980; Maehara et al., 1977; Sen & Herz, 1983). Therefore, by abolishing the production of endogenous IFN in the BAM cultures, the absolute protective effect exerted on the BAM declines, allowing viral replication and spread to resume. Thus, the protection seen at high levels of BoIFN-α may be (almost) exclusively due to exogenous IFN. Such an effect should be taken into account when IFN is being used clinically (Babiuk & Bielefeldt Ohmann, 1984).

As found in other species (Oud Alblas & van Furth, 1979; Zwilling et al., 1982) only a subpopulation of freshly isolated resident BAM from normal calves express Fc receptor activity, as revealed by rosette formation with Ig-sensitized erythrocytes. However, exposure to BoIFN-α for as short a time as 3 h enhances the percentage of erythrocyte-binding and -phagocytosing cells substantially, and increases the number of sensitized erythrocytes binding to each BAM. By assaying the cells in suspension, it was excluded that the increase was due merely to a steric effect following increased cell spreading (Babiuk & Rouse, 1978). Thus, the results indicate that the effect is due both to expression of Fc receptors on previously negative cells and to an increased number and/or avidity of the receptors on a particular cell. This is in agreement with recently published reports indicating that the enhancing effect of IFNs is due to an increase both in number and surface density of Fc receptors on cell membranes (Vogel et al., 1983; Yoshie et al., 1982).

Increased Fc receptor activity probably also contributed to the enhancement of ADCC expression of BAM, but cannot account for all of the increase, as judged by their different dose optimum and percentage increase (Fig. 1). The ADCC process is composed of an initial immune specific recognition and binding (Fc-mediated), followed by triggering of the macrophage and expression of cytotoxicity. Either or both of the latter steps may also be enhanced. However, exposure to BoIFN-α did not induce spontaneous cytotoxicity of the BAM as expressed against BHV-1-infected cells, such as has been reported in other species (Pace et al., 1983; Sen & Herz, 1983; Stanwick et al., 1980), but the treatment did enhance their cytostatic activity, especially after a relatively short exposure. The absence of spontaneous cytotoxicity may be a quality related to the target cell system rather than to the BAM. Thus, BAM can spontaneously kill cells infected with bovine parainfluenza 3 virus (Probert et al., 1977), suggesting that the virus-specific glycoproteins expressed on BHV-1-infected cells (Misra et al., 1981, 1982) do not have sufficient 'foreignness' to trigger BAM cytotoxicity, even though they had been primed by exposure to BoIFN-α (Pace et al., 1983).

Expression of extrinsic antiviral activity by macrophages is not limited to cytolytic functions, but can be conveyed also by a mechanism involving the host cell metabolism. This activity is expressed in vitro as an inhibition of viral plaque formation (Morse & Morahan, 1981; Rouse & Babiuk, 1975), probably via a cytostatic mechanism(s) (Keller, 1976; Morse & Morahan, 1981). Since BHV-1 replicates better in rapidly dividing cells, a cytostatic effect exerted on bronchiolar and alveolar epithelial cells by the BAM might be anticipated to serve to limit virus spread, especially early in infection, until specific immune mechanisms can come into effect. Thus, as IFN is produced early in an infection, the interactions between the resident AM and the IFN system may play a major role in the development of AM resistance in vivo (Forman et al., 1982a; Rodgers & Mims, 1982), and in the subsequent protection of the lung tissue proper. An effect on the AM may also be one of the mechanisms whereby exogenously applied BoIFN-α exerts its protective effect against a BHV-1 infection in calves, and increases their resistance to secondary bacterial infection (Babiuk & Bielefeldt Ohmann, 1984).

The authors thank Genentech, Inc. for providing the recombinant bovine alpha interferon, Greg Krakowski for assistance with the lavages and Bonnie Neufeld for typing the manuscript. Financial support was provided by grants from the Medical Research Council of Canada and Farming for the Future. H.B.O. was a post-doctoral fellow of the NATO Science Fellowship Programme (grants no. 23-03 51/81 and no. 23-03 38/82).
REFERENCES


IFN effect on virus-macrophage interactions


*(Received 21 March 1984)*