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EFFECTS OF PSEUDORABIES VIRUS INFECTION UPON CYTOTOXICITY AND ANTIVIRAL ACTIVITIES OF PORCINE ALVEOLAR MACROPHAGES

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Abstract—Alveolar macrophages (AM) infected with Pseudorabies virus (PRV) were compared to noninfected AM for cytotoxicity against foreign or transformed cells and production of interferon (IFN). Five PRV strains were used to infect AM including strains that are known to be highly virulent for pigs, i.e. strain 4892 and strain S-62 as well as strains that are regarded as mild or nonvirulent, i.e. BUK and Bartha. The multiplicity of infection ranged from 0.005 to 0.05 TCID₅₀/cell. The target cells in the cytotoxicity assays were either chicken red blood cells, PRV-infected vero cells, or human myeloblastoma cells (K562 cell line). For the production of IFN, AM cultures were treated with polyinosinic:polycytidylic acid (Poly I:C) diluted in tissue culture media at a concentration of 5 µg/10⁶ cells. Culture supernatants were collected at various times poststimulation and tested for antiviral activity using the Vesicular Stomatitis Virus replication inhibition test. Swine AM were able to lyse chicken red blood cells in an antibody-independent way but not in an antibody-dependent way, whereas lysis of PRV-infected vero cells was accomplished both ways. The cytotoxicity against chicken red blood cells was reduced in the PRV-infected AM as compared to noninfected cells, particularly in AM infected with virulent PRV strains. Specific ⁵¹Cr release values for AM infected with S-62 and 4892 strains were 14 and 19, while the noninfected AM had values of 36. Similarly, in the antibody-dependent cytotoxicity assay against PRV-infected vero cells there was no activity of AM against K562 cells. The production of IFN was readily stimulated with Poly I:C. The optimal time for supernatant collection was between 12 and 16 h poststimulation. The antiviral activity was abrogated by treatment of the supernatant with antiserum against human leukocyte IFN; it was therefore considered to be due to interferon-alpha (IFN α) released from the macrophages. The antiviral activity present in supernatants of PRV-infected AM was reduced compared to noninfected AM. The difference between AM cultures infected with virulent strains of PRV and noninfected AM cultures was statistically significant at $P \leq 0.025$. The results provide support to the premise that the role of AM in lung defense can be compromised by PRV infection.

Key words: Pseudorabies, Aujeszky's disease virus, cytotoxicity, alveolar macrophages, interferon alpha.

Résumé—Des macrophages alvéolaires infectés par le virus de la maladie d'Aujeszky (virus de la pseudo-rage, PRV en anglais) ont été comparés à des macrophages alvéolaires non infectés, au niveau de leur pouvoir cytotoxique pour des cellules étrangères ou transformées et au niveau de la sécrétion d'interféron. Cinq souches du virus PRV ont été utilisées pour infecter les macrophages alvéolaires, incluant aussi bien des souches connues pour être hautement virulentes chez le porc (souche 4892 et souche S-62) que des souches considérées comme faiblement ou non virulentes (BUK et Bartha). L'inoculation des cultures a été réalisée à des doses variables, allant de 0.005 à 0.05 TCID₅₀/cellule. Les cellules cibles utilisées dans les tests de cytotoxicité ont été soit des globules rouges de poulet, soit des cellules de la lignée cellulaire K526 (myéloblastome humain). Pour la production d'interféron, les cultures de macrophages alvéolaires ont été traitées avec de l'acide polyinosinique:polycytidylrique dilué dans le milieu de culture à une concentration de

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5 $\mu\text{g}/10^6$ cellules. Les surnageants de culture ont été prélevés à des temps variables après la stimulation et testés pour leur activité antivirale en utilisant le test d'inhibition de réplication du Virus de la Stomatite Vésiculaire. Les macrophages alvéolaires de porc ont été capables de lyser les globules rouges de poulet par une voie anticorps-indépendante mais pas par une voie anticorps-dépendante, alors que la lyse des cellules de la lignée vero infectées par le PRV a pu s'effectuer par les deux voies. Les macrophages alvéolaires infectés par le PRV se sont avérés moins cytotoxiques pour les globules rouges de poulet que les macrophages non infectés; particulièrement, pour les macrophages alvéolaires infectés par les souches virulentes du PRV (souches S-62 ou 4892), les valeurs de libération du ^{51}Cr étaient réduites, de 14 et 19 contre 36 pour les macrophages non infectés. Avec les cellules de la lignée vero infectées avec le PRV, le test de cytotoxicité anticorps-dépendante a conduit à des résultats similaires. Il n'y avait aucune activité cytotoxique des macrophages alvéolaires pour les cellules K526. La production d'interféron a été aisément stimulée par traitement des cellules avec de l'acide polycitidilique. Le moment optimal pour le prélèvement du surnageant s'est avéré être entre 12 et 16 h après la stimulation, et l'activité antivirale a été neutralisée par traitement des surnageants avec un antiserum anti-interféron de leucocyte humain: au vu de ces résultats, on doit considérer que l'activité antivirale est due à une production d'interféron-alpha par les macrophages. L'activité antivirale présente dans les surnageants des macrophages alvéolaires infectés par le PRV était moindre comparée à celle des macrophages alvéolaires non infectés; la différence d'activité entre les cultures de macrophages alvéolaires infectés avec des souches virulentes du PRV et les cultures de macrophages non infectés s'est avérée significative avec une probabilité $P \leq 0.025$. Ces résultats sont autant d'arguments pour considérer que le rôle des macrophages alvéolaires dans la défense pulmonaire peut être compromis par une infection par le virus de la maladie d'Aujeszky.

Mots clefs: Virus de la maladie d'Aujeszky, cytotoxicité, macrophages alvéolaires, pseudo-rage, interféron-alpha.

INTRODUCTION

Pseudorabies virus (PRV), also known as Aujeszky's disease virus, is a widespread swine pathogen. Clinical disease in swine varies depending on the age of the animal and the virus strain involved. In general, young pigs are more prone to acute disease with high mortality rates, whereas in mature animals, morbidity and mortality are reduced [1]. In growing-finishing pigs, the disease often includes respiratory problems caused either by the virus itself or by secondary pathogens such as *Pasteurella multocida* or *Mycoplasma hyopneumoniae* [2, 3]. The alveolar macrophage (AM) is considered to be fundamental in the defense of the respiratory tract particularly in the bronchioli and alveoli levels where the ciliated epithelium is lacking. The AM activities include phagocytosis and killing of bacteria, cytotoxicity against infected or transformed cells, and production of monokines [4, 5].

There is evidence that pseudorabies spreads widely in the respiratory tract from the tonsils down to the alveolar space; Narita *et al.* [6] reported finding PRV inside AM in tissue sections from intranasally-inoculated pigs. Additionally, *in vitro* experiments have shown that AM are susceptible to PRV, and that infected cells are less capable to carry out some of the killing activities that follow phagocytosis such as the oxidative burst [7]. Other important activities of the AM are independent from the phagocytic activity but are also essential in lung clearance, i.e. secretory activities and cytotoxicity against transformed, infected or foreign cells. The main objective of the work presented here was to evaluate the ability of PRV infected AM to lyse foreign, infected or transformed cells and to display antiviral activity. It is known that PRV infection eventually kills AM. It was previously reported that AM infected at m.o.i. of 3 were effectively evaluated as effector cells when used immediately after infection whereas at 24 and 48 h postinfection the cell viability was so reduced that it was difficult to compare the efficiency of PRV-infected or

noninfected AM as effector cells [8]. The evaluation of cytotoxicity and synthesis of IFN reported in this work were carried out immediately after infection, and the m.o.i.(s) used were chosen in a way that the time of assay would be coincidental with the time that replication and spread to noninfected cells were likely to occur. The target cells selected had all been used in cytotoxicity assays with swine leukocytes. The mechanisms of recognition and destruction that are active against each type of cell are presumed to be different from each other [9–11].

MATERIALS AND METHODS

Collection and culture of AM

Alveolar macrophages were collected from 6- to 9-week-old pigs by lung-lavage following the procedure previously described [12]. After separation of mononuclear cells by centrifugation in histopaque-1077 (SIGMA), the cells were $94 \pm 2\%$ macrophages as indicated by both GIEMSA differential staining and esterase nonspecific staining. Other cells present in the lung-lavage fluid were lymphocytes $3 \pm 2\%$ and other cells such as fibroblast 0.5–1%. The cells were cultured in RPMI-1640 plus 10% Newborn calf serum. Five PRV strains were used to infect AM. The PRV strains used included strains that are known to be virulent for swine, S-62 and 4892, and strains that are known for not producing clinical disease in pigs experimentally inoculated. The nonvirulent strains used were: BUK strain, Bartha strain. The field strain 3816 was also used. The origin and characteristics of all the strains have been previously published [13]. The virus stocks were produced in vero cells and kept at -70°C until used.

Target cells and hyperimmune sera used in the cytotoxicity assays

The cells used as targets for cytotoxicity assays were chicken red blood cells (CRBC), PRV-infected vero cells, and the cells K562 (Human myeloblastoma cell line, ATCC, Rockville, Md). In all cases, labeling was carried out by incubation with Na^{51}CrO (0.1 mCi per 10^7 cells) for 1–2 h at 37°C . Pig anti-CRBC hyperimmune serum was prepared by immunizing two 4-week-old pigs intramuscularly with CRBC (10^7 cells) mixed (1:1) with Freund's incomplete adjuvant. Pigs were boosted 2 weeks after the first immunization by administering 1000 cells diluted in sterile PBS intravenously. Sera collected 14 days after the second inoculation contained an anti-CRBC hemagglutination titer of 1:128. The pig anti-PRV was collected from a 10-week-old pig that had been vaccinated against PRV using a commercially available product (PRV-marker, Syntrovet, Lenexa, Kan.), then was challenged by intranasal instillation of 10^3 TCID₅₀ of PRV. Sera collected 14 days postchallenge had a neutralization titer against PRV > 256 .

Cytotoxicity assays

Alveolar macrophages that had been kept overnight in complete media in silicon coated tubes were assessed for viability by Trypan blue exclusion, viability was consistently $\geq 95\%$. The cells were concentrated by centrifugation and then infected or mock infected by resuspending the pellet of cells in a small volume of Hank's balanced salts solution (HBSS) containing the required amount of virus in order to achieve a multiplicity of infection (m.o.i.) of 0.05 TCID₅₀/cell. The mock infected cells were resuspended in a small volume of HBSS with no virus, after incubation at 37°C for 60 min, fresh HBSS was added,

the cells were pelleted again, the nonadsorbed virus was discarded, and the cells resuspended in RPMI + 2% NCS at the desired concentration ($10\text{--}30 \times 10^6/\text{ml}$). For the antibody-dependent assays, target cells were incubated with various dilutions of the hyperimmune sera, either against CRBC or against PRV for 60 min. Such incubation with sera dilutions was not required for the antibody-independent assays. Finally, the effector cells, either PRV-infected or noninfected AM, were added. In all cases, the incubation was for 16 h. There were several controls; the two assays based upon antibody-dependent cytotoxicity included control-wells with peripheral blood polymorphonuclear cells from age-matched pigs. The assays against PRV-infected vero cells included control-wells containing noninfected vero cells. The assays against K562 included control-wells with peripheral blood mononuclear cells from age-matched pigs. All assays included a set of wells containing only media and target cells that were later lysed for the 100% cytotoxicity value. Wells with no effector cells of any kind provided the value of spontaneous lysis. After the incubation period, supernatant samples (0.1 ml) were collected and counted in a gamma counter. The specific chromium release was calculated as described before [8]. Cells from five different pigs were used. Each test was repeated at least four times on different days. Due to the variations in the conditions of the assays, the results are presented as individual experiments.

Assessment of antiviral activity in the AM cultures supernatants

Preliminary experiments showed that AM cultures treated with polyinosinic:polycytidylic acid (Poly I:C) at $10 \mu\text{g}/10^6$ cells had antiviral activity in the supernatant collected 24 h poststimulation. The activity was measured by reduction of plaques caused by vesicular stomatitis virus (VSV) in vero cells. The antiviral activity was not detected in the supernatant of nontreated cultures, and it was still detected after dialysis against a pH 2.4 buffer for 18 h followed by dialysis with a pH 7.5 buffer. Since the antiviral activity was satisfactorily detected in primate cells, it was not limited to swine cells, thus it was considered to be due to type alpha interferon. In order to find out the optimum time for supernatant collection as well as whether PRV infection of AM will affect the synthesis of $\text{IFN}\alpha$, a quantitative assessment of antiviral activity was developed following the microtiter procedure described by Pedersen *et al.* [14]. Briefly, vero cell monolayers were plated in 96-well microtiter plates 6–14 h in advance in order to have a monolayer of cells already formed for the testing. The tissue culture media was removed from the monolayers and replaced with AM cultures supernatant diluted in tissue culture media $100 \mu\text{l}/\text{well}$. The cultures were incubated for 2 h at 37°C , then $100 \mu\text{l}$ of tissue culture media containing 20–50 TCID_{50} of VSV were added to each well. All samples were tested in quadruplicate. In all plates there were six wells with no supernatant sample, but virus as positive virus-induced cell destruction control, and six wells with no virus as cell control. The plates were incubated at 37°C for 24–36 h. The replication of VSV within cells was assessed with a dye uptake procedure described by Everitt and Wohlfart [15]. The plates were read in an ELISA reader using a 570 nm filter. The values of each set of wells were averaged, the values of each sample dilution were divided by the value of the positive control (only virus) and then plotted against dilution. It was noticed that the linearity of the curve was lost at very high or very low dilutions, but it was consistent in the range of dilutions from 1:5 to 1:20. The results are reported as the calculated value at 1:5 after plotting the values of four or more dilutions and calculating a regression line. The test was

used also to evaluate the antiviral activity present in supernatants of cultured AM from six pigs, those supernatants were evaluated before and after reacting with antibodies against human leukocyte IFN (SIGMA, St Louis, Mo).

The assessment of PRV infection upon synthesis of IFN was carried out by infecting cultures of AM made on 24-well plates with each one of the PRV strains previously mentioned at m.o.i. 5×10^{-3} or left as uninfected control. After the incubation period allowed for virus adsorption (1 h at 37°C), the virus inoculum was removed, and tissue culture media containing Poly I:C was added to each well 1 ml/well. A minimum of three cultures were infected with each strain. The supernatants from the cultures were collected at 20 h poststimulation and stored frozen until assayed. The potential presence of infectious PRV in the samples to be tested required neutralization of virus infectivity. It was accomplished by adding pig anti-PRV sera (final dilution 1:10) to the sample dilutions and letting it incubate for at least 2 h at 37°C before testing the sample on vero cells. The media containing pig serum that was used for dilutions was also used for the control (no sample) wells. The experiment was repeated on five different occasions using cells from different pigs. The significance of the difference between infected and noninfected cells was determined using an analysis of variance following the complete randomized block design where each pig was one block [16].

RESULTS

The cytotoxicity of AM against CRBC in the presence of hyperimmune serum was proportionally inverse to the serum dilution, and it was higher with the addition of negative sera indicating that it was not an antibody-dependent cytotoxicity (Table 1). The spontaneous cytotoxicity of AM against CRBC is presented in Fig. 1. It was noticed that PRV infected AM were less efficient at all effector to target ratios used. A total of five experiments were carried out. The counts per minute (CPM) ranged from 850 to 6500, while the total release ranged from 11,500 to 17,200. The AM infected with BUK were higher in activity as compared to the AM infected with S-62 or 4892, but still were below the chromium release values recorded by noninfected AM.

Table 1. Cytotoxicity of pseudorabies virus (PRV)-infected or noninfected alveolar macrophages (AM) against chicken red blood cells (CRBC)

| Dilution of serum | E:T ratio | Alveolar macrophages (AM) infected | | | |
|-------------------|-----------|------------------------------------|------|-----|------|
| | | S-62 | 4892 | BUK | None |
| 1:10 | | Neg* | Neg | Neg | Neg |
| 1:100 | 50 | Neg | Neg | 7 | 15 |
| 1:200 | | 5 | 9 | 17 | 23 |
| 1:5 | | Neg | Neg | Neg | Neg |
| 1:10 | 40 | Neg | Neg | Neg | Neg |
| 1:10 (-)† | | 18 | 16 | 34 | 49 |
| 1:20 | | Neg | Neg | Neg | Neg |
| 1:10 (-) | 20 | 14 | 19 | 29 | 36 |

*Specific chromium release values; negative indicates that the ^{51}Cr release values were lower than the values for spontaneous release.

†Except where indicated (-) the serum used was swine hyperimmune serum against CRBC.

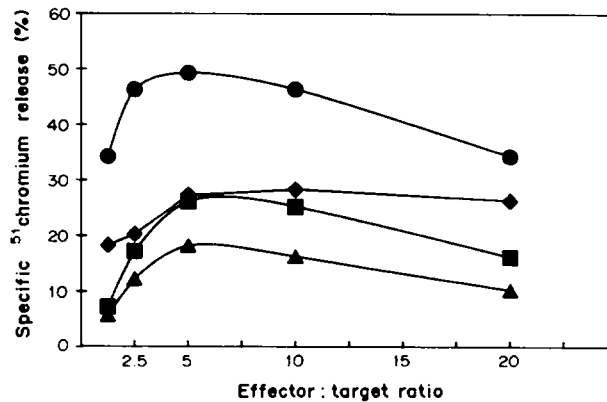


Fig. 1. Cytotoxicity of alveolar macrophages (AM) against chicken red blood cells (CRBC). The AM were infected with different strains of pseudorabies virus (PRV): ▲ 4892; ■ S-62; ◆ BUK; or left as ● noninfected control.

The cytotoxicity of AM against PRV-infected vero cells was clearly enhanced by antibodies as compared to the spontaneous cytotoxicity (Table 2). There was no cytotoxicity of AM against noninfected vero cells neither in the presence nor in the absence of PRV antibodies. The specific ⁵¹Cr release values recorded in these samples were below or equal to the values of the spontaneous release. In an assay carried out with an effector to target ratio of 50, the chromium release value of noninfected AM was 69, while PRV-infected AM had values of 42, 30, and 50 in cultures infected with S-62, 4892, and BUK, respectively. In one experiment, the hyperimmune sera was diluted up to 1:80, and no significant change in the results was noticed. The effector to target ratio variations produced some differences (Fig. 2). The cytotoxicity of AM against PRV-infected vero cells in assays carried out without antibodies was reduced compared to the ADCC. A difference in performance between PRV-infected AM and noninfected AM was better seen in assays carried out with high effector to target cell ratio, because at lower ratios the activity was undetectable. At the ratio of 40, the specific ⁵¹Cr release of noninfected cells was 14, while for cultures infected with S-62, 4892, or BUK the values were 4, zero, and 3, respectively. There was no cytotoxicity of AM against K562 cells.

Table 2. Cytotoxicity of pseudorabies virus (PRV)-infected or non-infected alveolar macrophages (AM) against PRV-infected vero cells in the presence or absence of specific antibodies

| Hyperimmune serum | E:T ratio | Alveolar macrophages (AM) infected | | | |
|-------------------|-----------|------------------------------------|------|-----|------|
| | | S-62 | 4892 | BUK | None |
| No | 50 | 10* | 13 | 14 | 18 |
| Yes | | 17 | 25 | NT | 46 |
| No | 50 | Neg | 2 | Neg | 14 |
| Yes | | 42 | 30 | 50 | 69 |
| No | 40 | 4 | Neg | 3 | 14 |
| No | 20 | Neg | Neg | Neg | 8 |
| Yes | | 8 | 12 | 20 | 27 |

*Specific chromium release values, negative indicates that the ⁵¹Cr release values were lower than the values for spontaneous release.

NT = Not tested.

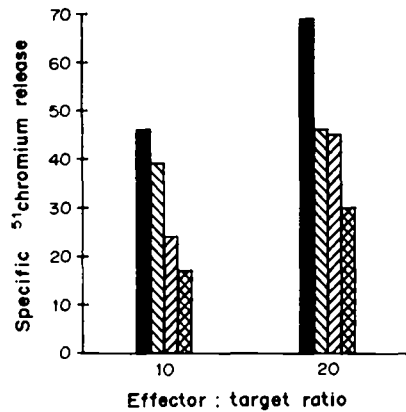


Fig. 2. Antibody-dependent cytotoxicity of alveolar macrophages (AM) against pseudorabies virus (PRV)-infected vero cells. Swine hyperimmune serum diluted 1:50 was used in all samples. The alveolar macrophages (AM) were infected with different strains of PRV: ▣ 4892, ▤ S-62, ▥ BUK, or left as ■ noninfected control.

The time course for collection of supernatants to be used for the evaluation of antiviral activity is presented in Fig. 3. It was noticed that the antiviral activity was first detected in supernatants collected 6 h poststimulation. The activity peaked at about 18 h poststimulation. The incubation of supernatant samples with antibodies reactive against human leukocyte IFN for 1 h prior to the test reduced the antiviral activity. Significant reduction, i.e. $\geq 10\%$ compared to nontreated controls, was accomplished with antibody dilutions of up to 1:100 (Table 3). The effect of PRV infection upon AM synthesis of IFN α is presented in Fig. 4. Supernatants from cultures infected with one of five PRV strains were compared to the supernatants from noninfected cultures. With the exception of Bartha strain and strain 3816, all the virus strains used were able to cause a reduction in the

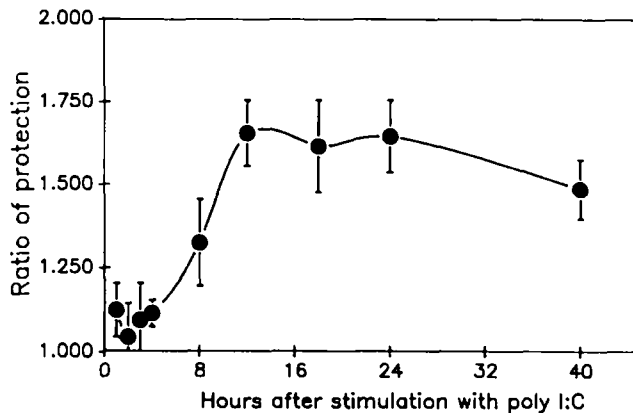


Fig. 3. Detection of antiviral activity in the supernatant of alveolar macrophages (AM) cultured with polyinosinic:polycytidylic acid (Poly I:C). The ratio of protection is the result of dividing the mean value of the sample wells by the mean value of the control-wells. It was adjusted to the value obtained at 1:5 (sample dilution). It was considered that values below 1.20 were negative. The graph shows the mean of values obtained with cells from three different pigs and the standard deviation.

Table 3. Antiviral activity of supernatants from polyinosinic:polycytidylic acid (Poly I:C) stimulated alveolar macrophages (AM) cultures after reacting with antibodies against human leukocyte interferon (IFN). Data represented as the index of protection at dilution 1:5. Values <1.20 were indicative of no protection

| Culture | Culture supernatant alone | Final dilution of the antibodies reactive against human IFN | | |
|---------|---------------------------|---|------|-------|
| | | 1:100 | 1:50 | 1:25 |
| A* | 1.82 | 1.68 | 1.34 | 1.52† |
| B | 1.61 | 1.39 | 1.12 | NT |
| C | 2.04 | 1.68 | 1.29 | NT |
| D | 1.37 | 1.26 | 1.04 | NT |
| E | 1.48 | 1.37 | 1.30 | 1.42 |
| F | 1.75 | 1.67 | 1.46 | 1.57 |

*Each culture was done with cells from one pig (total six pigs).

†All samples were tested with no serum and serum diluted 1:100 and 1:50 on the same assay. Some samples were tested again using dilutions of serum 1:50 and 1:25.

NT = Not tested.

amount of antiviral activity present in the supernatant. The difference in protection provided by cultures infected with S-62 and 4892 as compared to noninfected cultures was statistically significant at the $P < 0.05$ and $P < 0.025$, respectively.

DISCUSSION

The results presented here showed that swine AM were active against CRBC only in an antibody-independent assay. Peripheral blood polymorphonuclear cells showed positive cytotoxicity values indicating that the antibodies against CRBC were able to mediate ADCC, but AM were not active in such mechanism. Previously reported results showed that ADCC activity of lung cells from newborn pigs against CRBC was carried out by neutrophils present in lung-lavage fluid. In fact, there was a marked contrast in activity between samples from newborn and from 3-day-old pigs due to the shift in cell population; it was mainly neutrophils in newborn pigs [10]. In piglets as well as in mature animals,

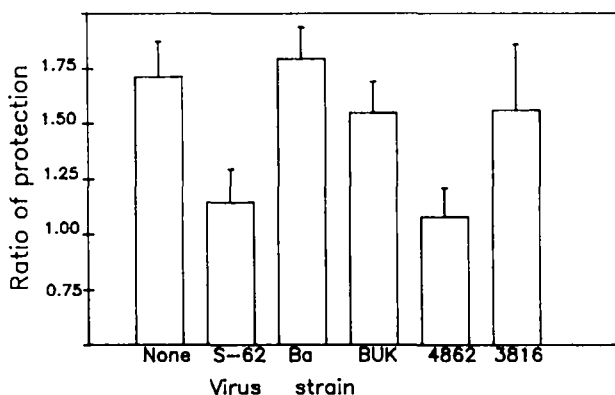


Fig. 4. Comparative assessment of interferon-alpha ($IFN\alpha$) production in alveolar macrophages (AM) cultures that were infected with pseudorabies virus (PRV) or left as noninfected controls. The supernatants were collected at 20 h poststimulation. The ratio of protection was calculated as explained in Fig. 3. The graph shows the mean of three experiments and the standard deviation.

the proportion of neutrophils in lung-lavage cells is very low. Antibody-dependent cytotoxicity against PRV-infected cells has been described with many types of effector cells, including lung-lavage cells [8, 11]. El-awar and Hanh [8] compared cells from young pigs (4–6 days old) to cells of mature pigs and found both populations were active in ADCC, and in both cases, PRV infection had an effect upon the activity. In our experiments, we compared three strains of virus, and it was noticed that similar to cytotoxicity against CRBC, the strains known to be virulent caused a reduction in the AM cytotoxicity. Antibody-independent cytotoxicity of AM against virus-infected cells has been described in other systems. Parainfluenza-3 infected calf kidney cells that were destroyed by lung-lavage cells from cattle [17]. Another system where such activity has been described was cells infected with infectious bovine rhinotracheitis virus [18]. In the case of pseudorabies, it is known that lymphocytes from lung-lavage were active against PRV-infected cells. Even though the activity was much lower as compared to peripheral blood lymphocytes, it was highly specific for virus-infected cells because the noninfected cells were not affected [19]. The fact that in our experiments some of the activity was carried out by lymphocytes cannot be ruled out, but it is unlikely to be the main active population because the proportion of lymphocytes in the effector cell population was very low. So it can be assumed that AM have a low rate of activity. Even though the infected AM had less activity as compared to the noninfected AM, the low values obtained in the specific release of chromium makes it difficult to have a reliable assessment of the effect of PRV infection upon this activity.

It is quite possible that AM in culture release a variety of cytokines and more than one could have antiviral activity. Some preliminary testing, therefore, was required. The antiviral activity detected in the supernatants of AM cultures was inducible by stimulation with Poly I:C, resistant to pH 2.0, not restricted to homologous cells, and it was reduced by treatment with antibodies against human leukocyte IFN which is known to cross react with swine IFN α [20]. Therefore, such activity was considered to be mainly due to IFN α . The activity most often associated with IFN, as well as the main criteria for evaluation, is the induction of resistance to virus infection. There are, however, many other activities in which IFNs are also involved. Phagocytosis of bacteria by AM and blood monocytes was enhanced after incubating the cells with homologous IFN [21], as well as the antibody-dependent cellular cytotoxicity of AM [18]. Porcine monocytes and porcine AM were rendered less permissive to African swine fever virus infection by treatment with bovine IFN [22]. It is clear that the production of IFN is an important component in the surveillance role carried out by the AM. It has been suggested that IFN has a role in the variations of virulence seen among herpes simplex virus (HSV) strains. For instance, Shimizu *et al.* [23] compared two HSV-1 variants. One was able to cause lethal infection of mice; the other did not cause lethal infection. The replication in culture of the latter was inhibited by anti-HSV antibodies, and it was highly sensitive to mouse IFN. In a different experiment, Brucher *et al.* [24] compared the replication of HSV in cells from susceptible or nonsusceptible mice. It was found that monocytes from susceptible mice supported virus replication more readily and produced less IFN as compared to monocytes from HSV-resistant mice. In the case of PRV, there was a report about attenuated strains being more sensitive to IFN and better IFN inducers as compared to virulent strains [25]. The experiments reported here, did not compare sensitivity to IFN, but there was indeed a significant difference between strains in the ability to lessen synthesis of IFN in cells stimulated with Poly I:C. The strains known to produce acute disease in swine were able

to reduce the synthesis of IFN, while the effect caused by other strains was not statistically significant.

The differences seen in either cytotoxicity or antiviral activity between PRV-infected and noninfected AM are unlikely to be solely due to a disparity of the viable cells in the cultures. Previous experiments have shown that in AM cultures infected at low m.o.i., the decrease in the number of viable cells is much higher in the period from 24 to 48 h as compared to the first 24 h postinfection (Iglesias *et al.*, unpublished results). This is perhaps due to the period required for dissemination of progeny virus. Such was the rationale for using low m.o.i. and to compare activities between PRV-infected and noninfected AM immediately after infection, considering that the differences in viability rates were $\leq 10\%$.

The results presented here show that PRV infection of AM with virulent PRV strains had a deleterious effect upon some of the defense activities carried out by alveolar macrophages. Experiments performed with cells retrieved from calves experimentally infected with bovine herpes virus 1, yielded similar results [26]. Knowledge about performance of swine AM after infecting pigs with PRV is lacking. Further research is needed in order to know the extent and severity of the AM impairment caused by PRV infection in naturally infected pigs.

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