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A PRO-INFLAMMATORY EFFECT OF FOOT AND MOUTH DISEASE VIRUS ON IMMUNE AND NON IMMUNE GUINEA PIGS

EFEITO PRÓ-INFLAMATÓRIO DO VÍRUS DA FEBRE AFTOSA EM COBAIAS IMUNES E NÃO IMUNES

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SUMMARY

The $O_{1Campos}$ strain of foot and mouth disease virus (FMDV) used as inducing agent in the pleurisy model was able to trigger a pro-inflammatory effect on normal and immune guinea pigs. The pro-inflammatory activity which was detected at two times of the pleurisy (24 and 48 hours) on normal guinea pigs was characterized only by mononuclear (MN) cell influx, during the first interval of the reaction and by edematogenic effect, MN and polimorphonuclear (PMN) leucocyte migration, at the last time of the reaction. The inflammatory reaction profiles recorded on immune guinea pigs (vaccinated with anti-$O_{1Campos}$ oil adjuvanted vaccine), both after 7 and 30 days post vaccination (pv) have showed, in both interval, lower intensities than that observed in normal guinea pigs, although in the 7 days PV guinea pigs the accumulations of total leucocytes and PMN were similar to that displayed by normal animals, after 48 hours of the reaction. Besides, on thirty days PV guinea pigs the FMDV induced a significant increase in volume of exudate and MN cell infiltration, after 24 hours, and all of the inflammatory parameters values dropped to normal levels, during the second interval of the reaction. It was found a negative association between the increase in serum neutralizing antibody titer, from 7 to 30 days PV and the intensities of pleural inflammatory parameters on the immune guinea pigs. The pleurisy test revealed itself feasible to evaluate the pro-inflammatory activity of FMDV.

UNITERMS: Foot-and-mouth disease virus; Pro-inflammatory effects; Immunity

INTRODUCTION

Little information is available about the inflammatory reactions produced during the course of infection caused by foot and mouth disease virus (FMDV). Leucocyte infiltration is known to occur in infected tissues, first with a predominance of polimorphonuclear (PMN) cells and later of mononuclear (MN) cells [18, 20, 23]. This cell infiltration is caused solely by the cell destruction induced by the virus[18].

There are no specific studies on FMDV such as those conducted on the Coxsackie B virus [11,22] and on togavirus, herpesvirus and poxvirus[4], in which the relevance of MN afflux was demonstrated in terms of limitation of the infection caused by one of these agents. The few reports available show the role of FMDV transport from blood to epithelial tissue by MN, macrophages and Langerhans cells after primary replications of the virus and viremia[9,23].

Furthermore, although the role of neutralizing antibodies in the protection against FMDV has been well established[5,11,17,21], the roles of the remaining defense mechanisms such as cell-mediated immunity and specially the delayed hypersensitivity reactions and of nonspecific protection processes such as inflammatory reactions, still need to be elucidated.

The objective of the present study was to investigate qualitatively and quantitatively the pro-inflammatory effect of FMDV using the pleurisy model in immune and in non-immune guinea pigs, at early and late phase of immune response.

MATERIAL AND METHOD

Animals

Female Duncan-Hartley guinea pigs weighting 700-750 g were used in the pleurisy test and in assay of protection against challenge, and families of albino Swiss suckling mice were used for FMDV propagation.

Virus

The virus used for the challenge the $O_{1CAMPOS}$ strain of FMDV which has been previously adapted to, and titrated in, guinea pigs. One ml of vesicular fluid collected from these guinea pigs contains $10^{5.6}$ DIGP50 (50% infectious and generalizing guinea pig doses).

The same viral strain from guinea pigs was adapted to and propagated in suckling mice in order to obtain more antigenic mass and it was titrated in guinea pigs again, and used as inducing agent in the pleurisy test at the concentration of 10^4 DIGP50/animal, diluted in PBS pH7.6, after being ground and centrifuged at 1600 g for 20 minutes at 4°C. Since the pleurisy-inducing viral suspension was not purified, a suspension of normal suckling mouse carcasses similarly processed and diluted in the same buffer was used as a control inducing agent. Normal or infected carcasses were stored in Vallée fluid at -70°C until they were processed.

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Oil FMDV Vaccine

The vaccine was prepared from a FMDV type O_1, strain Campos grown in BHK, clone 13 suspension cells, containing $10^{7.2}$ TCID50 and inactivated with binary imine ethylene[1]. An equal volume of oily adjuvant consisting of 9 parts Marcol 52(Exxon) and 1 part montanide 888(Sepicc) was added to this suspension and a simple aqueous emulsion in oil was prepared.

Experimental Protocol

A group of 64 guinea pigs received a vaccine dose of 0,25 ml each by subcutaneous route. Half of the animals were divided into two groups: one (N=8) was tested at challenge and other (N=24) in the pleurisy assay, both at 7 days post vaccination (pv) (early phase of immune response). The remaining animals were submitted to the same procedure, at 30 days pv (late phase of immune response). In addition, guinea pigs with no immunity to FMDV were tested both at challenge and pleurisy assay.

Challenge

The immune status of the guinea pigs was tested with challenge performed at 7 and 30 days pv by intradermoplantar inoculation of a vesicular fluid suspension containing 10^4 DIGP50 into the left hind paw.

Pleurisy Assay

The technique proposed by BECHARA at al.[2] (1986) was adopted along general lines. Non-immune and vaccinated guinea pigs were anesthetized with ethyl ether and injected intrapleurally with 1 ml of the test and control inducing suspensions. The animals were sacrificed 24 and 48 hours later (6 guinea pigs/group/interval of reaction) by prolonged exposure to ethyl ether and bled. The pleural cavity was exposed and washed with 3 ml PBS containing 50U heparin/ml and the inflammatory exudate was harvested for the determination of volume and number of total and specific leucocytes using a counting chamber and panchromically stained smears, respectively.

Anti-FMDV Antibody Titers

The serum samples obtained from guinea pigs submitted to challenge and to pleurisy assay were titrated for the determination of serum-neutralizing antibody concentration by the micro-colour test[17].

Statistical Analysis

A fully randomized experimental design was used and the means were compared by the Tukey test differences of $P > 0.05$ were considered significant[10].

For statistical analysis of the pleurisy data, volume and numbers of total leucocytes, MN and PMN, it was necessary to test heterogeneity of variances by the Bartlett test[7]. The data were transformed to $[\text{volume (ml)}]^{0.2}$ and $[\text{number of cells}]^{0.2} \times 10^6$ for later comparison of the means by the Tukey test, with the level of significance set at 5%[10].

RESULTS

FMDV-Specific Inflammatory Reaction in Non-Immune Guinea Pigs

FMDV injected into the pleural cavity of normal guinea pigs triggered an inflammatory reaction differing from that produced by the control inducing agent ($P<0.05$). From 24 to 48 hours, this reaction consisted of the elevation of MN during the first interval and persistence of elevated values of all of the inflammatory exudate parameters, i.e. volume, total number of leucocytes, numbers of MN and PMN (Fig. 1A, IB, 1C and 1D). In addition, the pleural cavity of noninjected guinea pig contained $1.47 \times 10^6 + 0.066$ total leucocytes, $1.39 \times 10^6 + 0.072$ MN and $1.06 \times 10^6 + 0.062$ PMN (values indicate mean of $(\text{number of cells})^{0.2} \times 10^6 + \text{S.E.}$).

FMDV-Specific Inflammatory Reaction in Immune Guinea Pigs

The virus-specific inflammatory response ($P<0.05$/pleurisy produced by control inducing agent) detected in guinea pigs immune to FMDV 7 days after vaccination, in regard to that triggered in normal guinea pigs, has demonstrated absence of edematogenic effect and lesser MN cell pleural infiltration, after 48 hours of the reaction, but it has showed similar leucocyte and PMN migration to pleural cavity, at the same time of the reaction. On the contrary, the FMDV inflammatory reaction on 30 days vaccinated guinea pigs showed only a significant increase in volume and MN number, though it presented a lower migration than that reported on 7 days vaccinated guinea pigs during a period of 24 hours of the reaction, taking into consideration that the pleural exudate parameter values dropped to normal levels after a 48 hours reaction period (Fig. 2A, 2B, 2C and 2D).

Humoral immune Response and Relative Protection Against Challenge

The protection against generalized vesicular lesions was 100% in immune guinea pigs both 7 and 30 days pv, with an increase in serum-neutralizing antibody titers from the first to the second pv interval (Tab. 1).

DISCUSSION

The importance of MN leucocyte recruitment in virus infected tissues has been pointed out[4,22]. However, few data are available with respect to the inflammatory reaction induced by the FMDV, especially in terms of the dynamics of leucocyte infiltration. Only the histopathological features of the skin or mucosae affected by the virus have been described. In these reports, leucocyte afflux was understood to be an event caused solely by cell destruction[18,20,23].

However, histological techniques are not always adequate for the study of the dynamics of leucocyte infiltration **in vivo**[12]. Thus, when FMDV-induced pleurisy model was introduced in the present study, it was observed that the virus does indeed have an edematogenic and chemotactic effect, as shown in particular by the increase in the MN number and volume of the exudate formed 48 hours after the induction of a reaction in non-immune animals. During a period of 24 hours it was only possible to distinguish a greater MN afflux as an FMDV-

specific inflammatory response, whereas the other inflammatory parameters did not differ from those recorded for the pleurisy induced by the control agent.

The pro-inflammatory activity of viruses has been previously demonstrated by the production of chemotactic factors for PMN and MN in the allantoid fluid of chicken egg embryos and in the supernatant of cell cultures infected with Newcastle disease virus and mumps virus, respectively[21]. The chemotaxis detected in the study cited was clearly a consequence of cell infection, whereas this did not seem to be the case in the pleurisy induced by FMDV since in this test virus is introduced at a site that is not a target for its replication. This fact strengthens the idea that the interaction of FMDV with normal cells of the pleural cavity, and in particular the leucocytes present there, may have triggered the inflammatory process in question, as is the case for the action of bacterial lipopolysaccharides on macrophages residing in the peritonium[8].

Another viral pro-inflammatory effect **in vivo**, characterized by infiltration of MN leucocytes has also been reported in a study using mice inoculated intracerebrally with a togavirus. In addition, the most intensive recruitment of leucocytes to the cerebrospinal fluid coincided with the beginning of a pronounced drop of infectious viral titer in this fluid at a time when antiviral antibodies were still undetectable in the serum of these animals[4].

In contrast, the inflammatory process induced by FMDV on vaccinated animals varied according to the phase of immunity. At 7 days pv, the profile of FMDV inflammatory reaction presented characteristics similar to those of non-immune guinea pigs, at 48 period of the reaction, though it displayed a lower intensity of MN cell recruitment and the absence of edematogenic activity. On the contrary, on 30 days pv guinea pigs, there was only a significant increase in volume and MN number promoted by FMDV after 24 hours of the reaction, indicating a possible occurrence of a cito-mediated immune response which contributed to leading the inflammatory reaction induced by the FMDV to a resolution, after a 48 hours interval. Furthermore, with the increase in serum-neutralizing antibody titers from 7 to 30 days pv, a negative immunomodulator effect appears to have occurred, causing partial inhibition of the FMDV-specific inflammatory process. This hypothesis is supported by the previous report that the increase in anti-togavirus antibody titer in mice challenged intracerebrally produced a decrease in leucocyte infiltration into cerebrospinal fluid[4].

The present results may also mean that the inflammatory reaction induced by FMDV in guinea pigs in the initial stage of immunity may be of importance, together with the process of humoral immune response, in favoring an amplification of viral opsonization and of the blockade of viral infectiousness, as indirectly shown by challenge test. In this respect, there is a suspicion as well as direct and indirect evidence that opsonization or the inflammatory reactions that favor its development represent other mechanisms of protection in addition to those of neutralization by antibodies against the infection produced by FMDV or by another picornavirus such as Coxsackie B virus[13,14,15,16,19].

Thus, the present study demonstrated the feasibility of the use of the pleurisy model to investigate the pro-inflammatory activity of FMDV and other viruses, especially in terms of the edematogenic and chemotactic activities. The protective role of MN recruitment against FMDV in immune animals was also infered, during the early and late phase of immunity.

RESUMO

A estirpe $O_{1CAMPOS}$ do vírus da febre aftosa (VFA) usada como agente indutor do teste de pleurisia foi capaz de desencadear um efeito pró-inflamatório em cobaias normais e imunes. A atividade pró-inflamatória do VFA, detectada em dois intervalos de pleurisia (24 e 48 horas) foi demonstrada, somente por quimiotaxia de leucócitos mononucleares (MN), no primeiro intervalo e por efeito edematogênico, migração de MN e polimorfonucleares (PMN), no último intervalo de reação. Os perfis de reação inflamatória induzida pelo VFA em cobaias imunes (imunizadas com vacinas oleosas anti-$VFAO_{1Campos}$), aos 7 e aos 30 dias pós-vacinação (PV) apresentaram intensidades mais baixas do que as observadas em cobaias normais, embora nas cobaias com 7 dias de vacinação a quimiotaxia de leucócitos totais e de PMN tenha sido similar àquela encontrada nos animais normais, no intervalo de 48 horas de reação. Ademais, nas cobaias com 30 dias PV, o VFA induziu um aumento significante no volume de exsudato e na infiltração de MN, no intervalo de 24 horas, sendo que os valores de todos os parâmetros do exsudato inflamatório caíram a níveis normais, no segundo intervalo de reação. Nas cobaias imunes foi observada uma associação negativa entre o aumento no título de anticorpos soro-neutralizantes, de 7 para 30 dias PV e as intensidades dos parâmetros inflamatórios pleurais. O teste de pleurisia revelou-se um procedimento adequado para avaliar a atividade pró-inflamatória do VFA.

UNITERMOS: Virus da febre aftosa; Efeito pró-inflamatório; Imunidade

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TABLE 1

Serum-neutralizing anti-FMDV antibody titer and relative protection against challenge in non-immune and immune guinea pigs 7 and 30 days after vacination.

Phase of immunity	Serum-neutralizing antibody titer	Percent protection against challenge no. protected/no. challenged
non-immune	0.4950 + 0 (n=14) [b]	0 (0/8)
7 days pv	1.2330 + 0.1858 (n=14)	100 (8/8)
30 days pv	2.2358 + 0.3030 (n=14)	100 (8/8)

a - The titer is expressed as mean ± SD log 10

b - n - corresponds to the number of animals tested; of these, 6 were from the pleurisy test and 8 from the challenge test.

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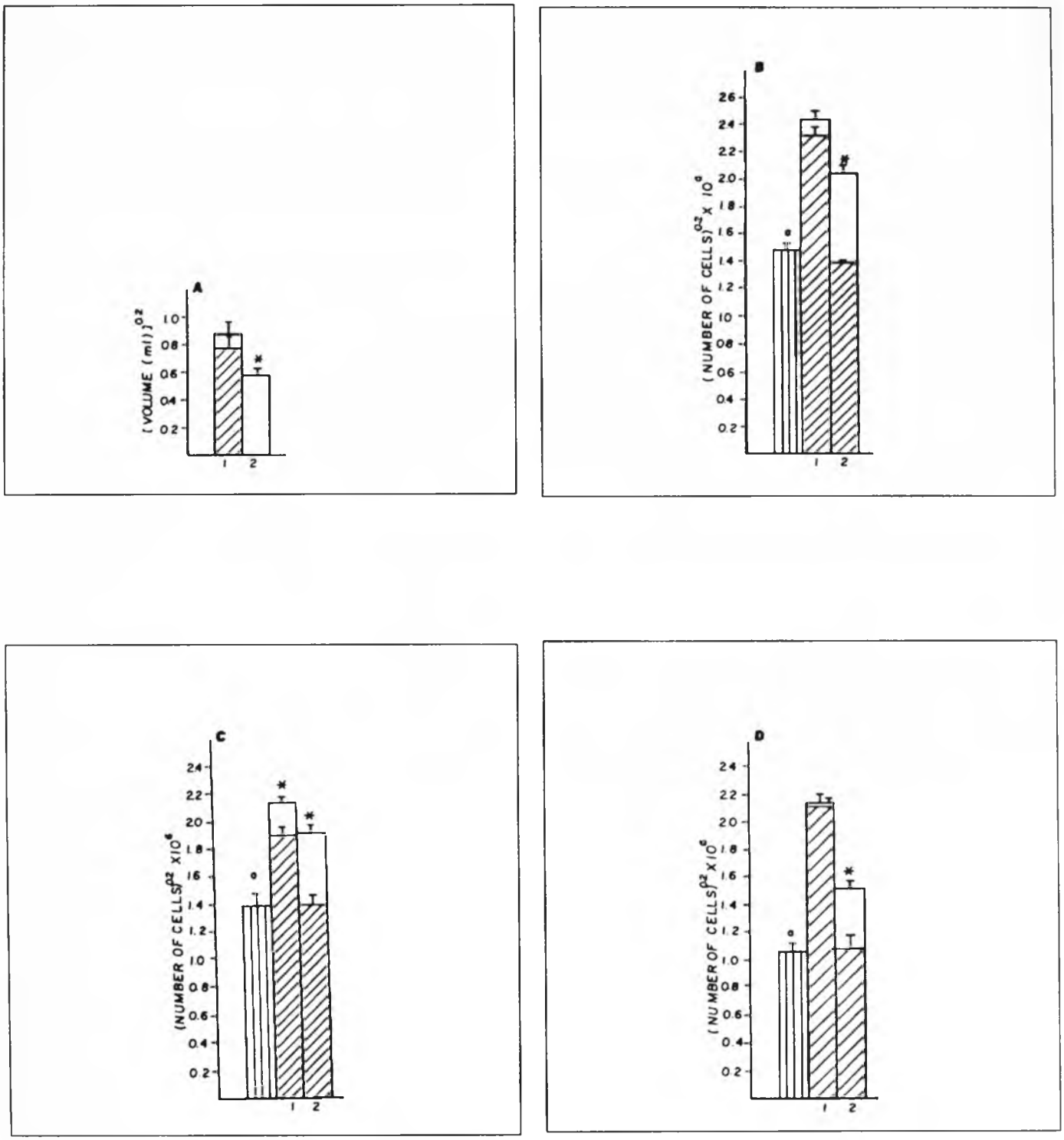
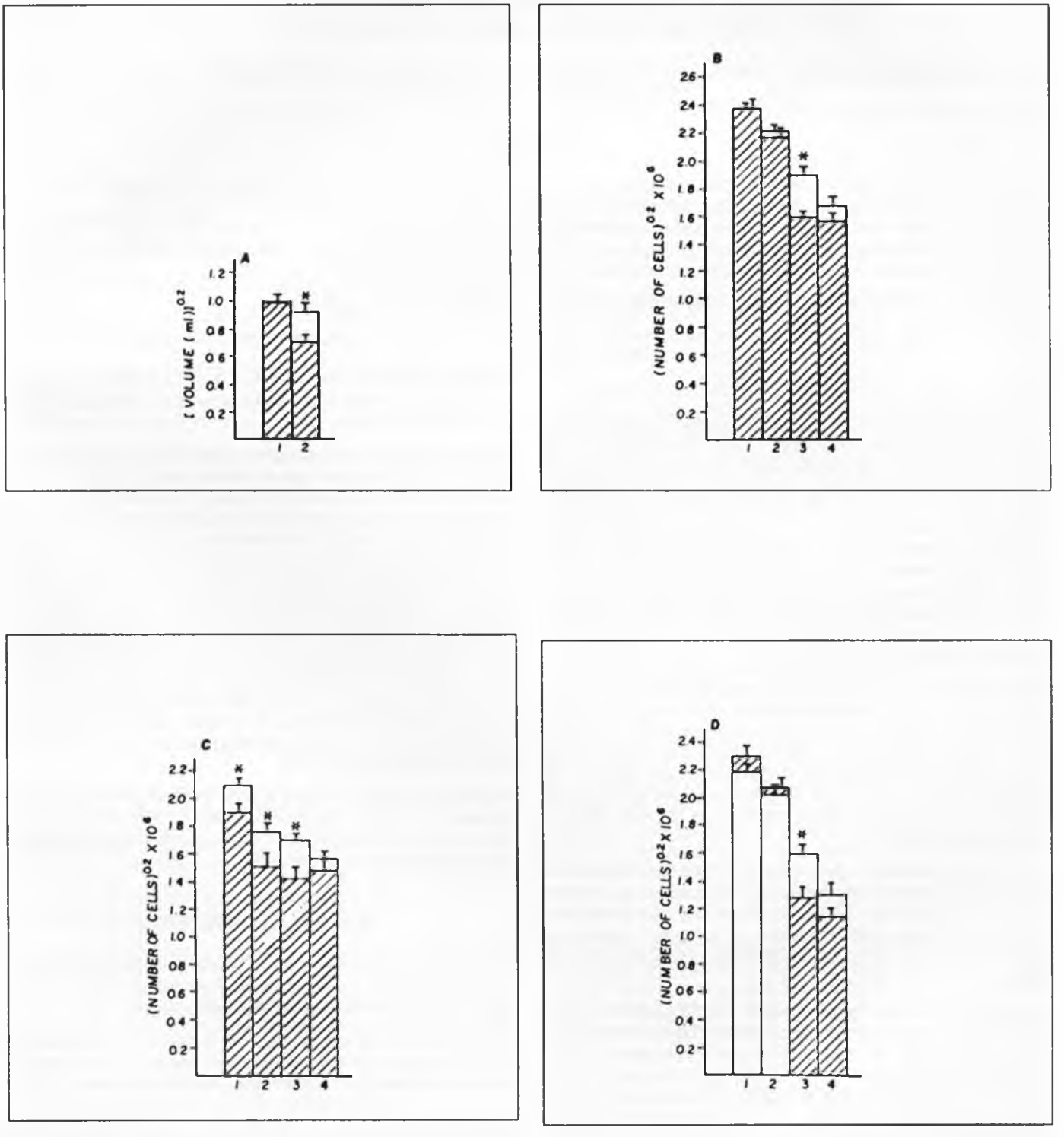


Figures 1A, 1B, 1C AND 1D - Effects of foot and mouth disease virus on the volume of exudate (Figure 1A) and on the migration of total leucocytes (Figure 1B), mononuclear (Figura 1C) and polymorphonuclear (Figure 1D) cells during a period of 24 (columns 1) and 48 hours (columns 2) of pleurisy produced by test-inducing agent (light columns) and by control-inducing agent (hatched columns) in normal guinea pigs. The numbers of resident cells in the normal pleural cavity are presented (columns with vertical lines).

* Represents statistically significant differences ($P<0,05$) between the inflamation produced by the test-inducing agent and by the control-inducing agent.

a Represents statistically significant differences between the number of resident cells in the normal pleural cavity and the cells that migrated in response to the FMDV stimulus.



FIGURES 2A, 2B, 2C AND 2D - Effects of foot and mouth disease virus on the volume of exudate (Figure 2A) and on the migration of total leucocytes (Figure 2B), mononuclear (Figure 2C) and polimorphonuclear (Figure 2D) cells, during a period of 24 (columns 1 e 2) and 48 hours (columns 3 e 4) of pleurisy produced by a test-inducing agent (light columns) and a control-inducing agent (hatched columns) in immune guinea pigs after seven days P.V. (columns 1 and 3) and thirty days P.V. (columns 2 and 4).

* Represents statistically significant differences ($p<0.05$) between the inflammation produced by the test inducing agent and by the control inducing agent.