Enrichment of coronavirus-induced interferon-producing blood leukocytes increases the interferon yield per cell: a study with pig leukocytes

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SUMMARY

Porcine peripheral blood mononuclear cells, which secrete IFNα in response to a coronavirus, transmissible gastroenteritis virus, were detected by a filter immunoplaque assay (ELISPOT). IFNα-producing cells (IPC), which are present at a low frequency in the blood, could be enriched up to 100-fold by sequential depletion of plastic-adherent cells and cell fractionation on metrizamide density gradients. IPC were present in the non-adherent low-density cell subpopulation. Cell selection experiments using antibody (Ab)-coated immunomagnetic beads revealed that porcine IPC could be positively selected by anti-CD4 or -SLA-class-II Ab, but not by anti-CD2 or -CD8 Ab. The estimated IFN yield per IPC was found to increase when IPC were assayed at higher concentrations. These data suggest that IPC represent a unique and distinct cell population in the blood, which could secrete higher amounts of IFN following its accumulation at a site of viral infection.

Key-words: Coronavirus, Leukocyte, Interferon alpha, Transmissible gastroenteritis virus; IPC, ELISPOT, Immunobeads, PBMC, mAb.

INTRODUCTION

Peripheral blood mononuclear cells (PBMC) from several mammalian species are capable of secreting interferon-α (IFNα) *in vitro* following brief exposure to non-infectious viral structures, such as inactivated virus particles or glutaraldehyde-fixed virus-infected cell monolayers (Lebon *et al.*, 1982; Capobianchi *et al.*, 1985; Kurane *et al.*, 1986). These findings suggest that this IFNα-induction mechanism does not require

viral replication in IFNα-producing leukocytes, but rather, may be the consequence of membrane interactions between IFNα producer cells and viral proteins present at the surface of virus particles or virus-infected cells. Indeed, several reports indicate that glycosylated viral proteins may be the essential triggers for IFNα induction (Ito *et al.*, 1978; Lebon, 1985; Charley *et al.*, 1991; Laude *et al.*, 1992; Capobianchi *et al.*, 1992). Cells which produce IFNα in response to non-infectious viral structures (IPC, or IFNα-

producing cells; also referred to as natural IFNα-producing cells (Ito *et al.,* 1981)), though very infrequent among PBMC, are highly efficient producers of IFNα (Gobl *et al.,* 1988). IPC, which circulate throughout the body and secrete high amounts of IFN without being infected by the inducer virus, are likely to play a major role in early non-specific antiviral host defences. Although the precise nature of IPC is not fully elucidated, it has been shown that human IPC are non-phagocytic null cells, lacking the cell surface markers specific of B, T or NK cells or monocytes, but expressing MHC class II molecules and low levels of CD4 antigens (reviewed in Charley and Laude, 1992). Identification of the cells responsible for producing IFNα was recently carried out using several technical approaches: cells containing mRNA for IFNα were identified by RNA-RNA *in situ* hybridization (Gobl *et al.,* 1988), whereas immunocytochemistry with monoclonal or polyclonal anti-IFNα antibodies detected cells containing cytoplasmic IFNα (Sandberg *et al.,* 1989; Feldman and Fitzgerald-Bocarsly, 1990). A filter immunoplaque assay (ELISPOT) was also used to visualize IFNα-secreting cells (Cederblad and Alm, 1990). Assuming that most IPC were detected by such techniques, and that the different IFN subtypes present in the supernatants of induced leukocytes were produced by the same cells, it was suggested that the yield of IFN per IPC could be estimated from the amount of IFN in supernatants and the IPC number per culture. Thus, herpes simplex virus(HSV)-induced human IPC were found to secrete between 0.5 and 2 units of IFNα per cell (Cederblad and Alm, 1990).

In order to analyse in more detail the nature of human IPC, several approaches were used to select these cells, including separation on density gradients, sequential cell depletion or flow cytometry cell sorting with anti-leukocyte monoclonal antibodies (mAb) (Fitzgerald-Bocarsly *et al.,* 1988; Sandberg *et al.,* 1990). IPC which responded to HSV- or to cytomegalovirus (CMV)-infected cells were recovered in the low density fraction of metrizamide gradients, but were found to be functionally distinct from dendritic cells (Chehimi *et al.,* 1989).

In previous studies, we have shown that porcine PBMC can secrete IFNα following exposure to non-infectious transmissible gastroenteritis virus (TGEV) (Charley and Laude, 1988), an enteropathogenic coronavirus which induces high IFN production in infected animals (La Bonnardière and Laude, 1981). In the present report, we have enriched TGEV-induced IPC by using cell depletion or cell-sorting procedures with specific mAb or by cell fractionation on discontinuous density gradients. The IPC were monitored by ELISPOT assay for secreted IFNα, and by IFN bioassay in culture supernatants. From these data, the IFN yield per IPC was estimated in the different cell fractions obtained and was found to vary with the degree of IPC enrichment.

MATERIALS AND METHODS

PBMC

Porcine PBMC were obtained from heparinized blood by Ficoll density centrifugation on "MSL" (density 1.077, Eurobio, Paris). PBMC were suspended in RPMI-1640 medium supplemented with 10 % foetal calf serum (FCS) and antibiotics. Porcine PBMC were then used directly in IFN induction or were depleted of plastic-adherent cells as follows: PBMC were resuspended in RPMI-1640 medium supplemented with 20 % FCS at a concentration of $2\text{-}5 \times 10^6$ cells/ml and incubated for 90 min at 37°C in tissue culture flasks. "Plastic-non-adherent cells" were then collected by shaking the flasks. In some

CMV	=	cytomegalovirus.
CSF	=	colony-stimulating factor.
ELISPOT =		solid-phase enzyme-linked immunospot.
FCS	=	foetal calf serum.
HSV	=	herpes simplex virus.
IFN	=	interferon.

IPC	=	IFNα-producing cell.
mAb	=	monoclonal antibody.
PBMC	=	peripheral blood mononuclear cell.
PBS	=	phosphate-buffered saline.
ST	=	swine testis.
TGEV	=	transmissible gastroenteritis virus.

experiments, phagocytic cells were depleted by carbonyl iron ingestion before Ficoll density centrifugation (Charley and Lavenant, 1990).

Anti-porcine leukocyte mAb (Lunney and Pescovitz, 1987)

mAb MSA4 (anti-CD2), 76-7-4 (anti-B), 74-12-4 (anti-CD4), 74-22-15 (anti-macrophages-granulocytes) and MSA3 (anti-SLA-class-II) were kindly provided by J. Lunney (USDA, Beltsville, MD, USA); mAb 295/33 (anti-CD8) was kindly provided by U. Koszinowski (Tübingen, FRG). mAb TH22A5 (anti-SLA-class-II) was purchased from VMRD (Pullman, WA, USA). These antibodies were used as ascitic fluids.

Treatment of cells with mAb and complement

Cells were incubated for 30 min with mAb and complement at 37°C as described previously (Charley and Lavenant, 1990): briefly, one-month-old rabbit serum served as a source of complement at a final dilution of 1/9 and mAb were used at 1/200. The percentage of dead cells was determined by trypan blue exclusion and cells were readjusted to the final viable cell concentration before use.

Separation of cells on Percoll and metrizamide density gradients

Separation of plastic-non-adherent cells on Percoll gradients was performed as described by Fitzgerald-Bocarsly *et al.* (1988): Percoll (Pharmacia, Uppsala, Sweden) was adjusted to 302 mOs/kg H_2O by the addition of 10× concentrated PBS and five concentrations of Percoll in RPMI 10 % FCS (40 %, 45 %, 50 %, 55 % and 60 %) were layered in 15-ml conical tubes; $12\text{-}17 \times 10^7$ cells were layered onto gradients which were centrifuged at 550 g for 60 min. Cells from interfaces and pellet were collected, washed and stored overnight at 4°C before induction.

Separation of non-adherent cells on metrizamide gradients was performed as described by Knight *et al.* (1986): four concentrations of metrizamide (Sigma, St Louis, USA) in RPMI 20 % FCS (14.2 %, 15 %, 16 % and 17 %) were layered in 15-ml conical tubes; plastic non-adherent cells (3 ml at $2\text{-}10 \times 10^7$/ml in RPMI 20 % FCS) were layered on top of gradients and the tubes were centrifuged at 550 g for 60 min. Cells from the interfaces and from the pellet were collected, washed twice and stored overnight at 4°C before induction.

Separation of cells with immunomagnetic beads

The immunomagnetic indirect separation technique using "M-450 Dynabeads" coated with sheep anti-mouse IgG Ab (ref. 1101, from Dynal, Oslo) was performed as described in instructions for use. Briefly, cells were pretreated with different anti-porcine cell subset mAb (diluted at 1/100) as indicated in the results for 30 min. After washing, pretreated cells were mixed with coated Dynabeads, at a ratio of 8 beads per cell. The mixture was gently vortexed and centrifuged to obtain optimal rosette formation. Following 30 min of incubation at 4°C with agitation, rosette-forming cells (the rosette-positive cell fraction) and particles were removed with a magnet, whereas the rosette-free suspension was decanted. Both positively and negatively selected cell fractions were washed and stored at 4°C before induction.

Surface marker analysis

The proportion of different leukocyte subsets and SLA-class-II^{+} cells in the fractions prepared on metrizamide gradients was analysed by indirect immunofluorescence using the anti-porcine leukocyte mAb described above. Briefly, cells were incubated at 4°C for 45 min with 1/10 dilutions of appropriate mAb, washed and resuspended in fluoro-isothiocyanate-conjugated anti-mouse antiserum (Biosys, Compiègne, France; 1/10). After 45 min at 4°C and 3 washes, at least 300 cells were scored under the microscope.

Production of virus

The high-passage Purdue-115 strain of TGEV was used as a virus source. The procedures for virus propagation in the pig kidney cell line PD5 and titration of infectivity in the swine testis (ST) cell line were reported previously (Laude *et al.*, 1992).

IFNα induction

After the various separation procedures described above, cells were stored overnight at 4°C in RPMI 20 % FCS. Mononuclear cells were then induced to produce IFNα by incubation with TGEV in 96-well microplates as follows: cell preparations were incubated in duplicates at usual final concentrations of 4×10^6, 2×10^6 and 1×10^6 viable cells per ml (except for metrizamide- or Percoll-separated cells which were incubated at 4×10^5, 2×10^5 and 1×10^5 cells/ml), in a total volume of 200 μl of RPMI 10 % FCS containing 2×10^4 plaque-forming units of TGEV. After 8 h at 37°C, the induced cells were resuspended and 100 μl from each well were trans-

ferred to nitrocellulose-bottomed microplates for the ELISPOT assay (see below). The other 100 µl of induced cells were further incubated overnight at 37°C for IFN bioassay.

ELISPOT (solid-phase enzyme-linked immunospot) assay

The ELISPOT assay was performed as described (Cederblad and Alm, 1990): nitrocellulose-bottomed 96-well filtration plates (ref. Mahan 4550 from Millipore, Guyancourt, France) were coated with anti-porcine IFNα mAb (mAb K9, described by Lefèvre et al. (1990) and L'Haridon et al. (1991)) at a final concentration of 0.8 µg/ml in PBS. The plates were then fixed with 0.05 % glutaraldehyde and blocked with 3 % glycine in PBS. TGEV-induced cells were added to the wells and incubated overnight at 37°C. Following extensive washing, the plates were then incubated with peroxidase-conjugated anti-porcine IFNα mAb F17 (Lefèvre et al., 1990 and L'Haridon et al., 1991) at a final concentration of 2 µg/ml for 1 h at 37°C in 5 % bovine serum albumin and 0.05 % Tween PBS. After washing, each well received 0.1 ml substrate solution consisting of di-aminobenzidine with perhydrol. The plates were finally dried and spots were counted under a macroscope. The frequency of IPC was calculated as the mean number of spots divided by the total PBMC number in each well of the induction cultures.

IFN bioassay

Serial $\log_3$ dilutions of supernatants from induced cells were assayed for IFN on bovine MDBK cells using vesicular stomatitis virus as a challenge (La Bonnardière and Laude, 1981). Our internal standard porcine IFNα was included in each assay. This standard was calibrated on MDBK cells with the human international reference IFN B69/19 (NIH, Bethesda, MD, USA). In our results, 1 U was equivalent to 1 IU of human IFN. The estimated amount of IFN produced by each IPC was calculated from the titre of IFN (units) in induction culture supernatants and the IPC numbers per culture.

RESULTS

Sequential enrichment of TGEV-induced IPC

Porcine PBMC obtained from Ficoll Hypaque gradients are induced to produce IFN following incubation with TGEV. The IPC, as monitored by the ELISPOT assay, were found to be infrequent among PBMC (1 per 10^4; table I). When PBMC were depleted of plastic-adherent cells and/or of phagocytic cells before viral induction, the IPC frequency was 2- to 3-fold increased in the non-adherent, non-phagocytic cell fraction (table I). Further treatment of non-adherent cells with different anti-leukocyte, mAb and complement, before viral induction, showed that anti-CD4 mAb with complement almost completely depleted IPC (table II). Similarly, IPC were positively selected by anti-CD4-coated immunomagnetic beads (table III).

In these experiments, mAb directed to B cells, to $CD2^+$ or $CD8^+$ cells or to SLA-class-II$^+$ cells did not abolish IPC in the presence of complement (table II), nor could they positively select IPC with immunobeads (table III). The frequency of IPC was even increased following complement-mediated depletion of $CD8^+$ cells (table II). These data indicated, therefore, that when non-adherent cells were treated with mAb before virus induction, IFNα-secreting cells could

Table I. Effect of depletion of adherent and phagocytic cells on IFNα production.

Cell population	Proportion of cells (%)	No exp.	IPC frequency ($\times 10^{-5}$)	IFN yield (units per IPC)
PBMC	100	28	10.8 ± 3.1	3.1 ± 3.6
Non-adherent cells	79.6 ± 4.4	28	20.2 ± 7.3	3.6 ± 4.4
Non-phagocytic cells	83.1 ± 9.2	4	20.9 ± 21	3.5 ± 6
Non-adherent and non-phagocytic cells	70.3 ± 10.1	4	30.6 ± 25.9	5.1 ± 5.3

The different cell populations (2×10^6/ml) were induced for 8 h with TGEV. The IPC frequency was subsequently determined by the ELISPOT assay and the amount of IFN (units) in cell supernatants was determined by IFN bioassay.

Table II. IPC frequency in cell populations treated with mAb and complement before virus induction.

	Specificity	No. exp.	Viable cells (%)	IPC frequency ($\times 10^{-5}$)
Complement alone	—	7	92.9 ± 3.2	15.1 ± 12.4
76-7-4	B cells	4	88.3 ± 4.5	21.7 ± 35.5
MSA4	CD2	4	57.8 ± 7.9	22.6 ± 34.4
74-12-4	CD4	6	74.7 ± 3.7	0.8 ± 0.4
295/33	CD8	5	60.4 ± 8.7	33.0 ± 34.9
TH22A5	SLA class II	4	73.5 ± 3.2	25.4 ± 43.7
MSA3	id.	4	79.5 ± 4.6	23.5 ± 39.3

Non-adherent cells pretreated with mAb and complement were resuspended to the final concentration of 2×10^6 viable cells/ml for virus induction.

Table III. IPC frequency after cell selection with immunomagnetic beads.

			Immunomagnetic selection			
			Positive		Negative	
mAb	Specificity	N° exp.	Percent cells	IPC frequency ($\times 10^{-5}$)	Percent cells	IPC frequency ($\times 10^{-5}$)
Control medium		7	11.9 ± 2.4	6.2 ± 7.1	88.1 ± 2.4	34.9 ± 27.6
76-7-4	B cells	1	18	2.5	82	20
MSA4	CD2	1	43	5.2	57	35
74-12-4	CD4	6	31.3 ± 6.8	47.3 ± 31.8	68.7 ± 6.8	3.9 ± 4.0
295/33	CD8	3	33.8 ± 22.5	1.8 ± 2.5	66.2 ± 22.5	34.1 ± 32.5
TH22A5	SLA class II	3	26.6 ± 16.4	10.7 ± 35.7	73.4 ± 16.4	51 ± 103.5
MSA3	id.	5	27.3 ± 17.7	10.2 ± 21.4	72.7 ± 17.7	28.4 ± 35.5

be characterized as non-T, non-B, CD8⁻ but CD4⁺ cells, partly confirming previous observations of the effects of complement-mediated cell depletion on IFN production (Charley and Lavenant, 1990). However, a surprising result in the present experiments was that IPC monitored by ELISPOT were mostly SLA-class-II-negative, in contrast with our previous results on IFN production by mAb-treated leukocytes (Charley and Lavenant, 1990). Such a discrepancy might be explained by the results of the following experiments in which immunomagnetic beads/cell separations were performed either before or after virus induction: table IV shows that a much higher proportion of porcine IPC was recovered in the positively selected cell fraction when anti-CD4- or -SLA-class-II-coated beads

were used after virus induction instead of before virus induction.

An alternative approach to selecting IPC was to fractionate PBMC on discontinuous density gradients using Percoll or metrizamide. When plastic non-adherent cells were fractionated onto Percoll gradients, cells from the low-density fraction (45 % Percoll) corresponding to less than 15 % non-adherent cells were 10- to 17-fold enriched for IFNα-secreting cells (table V). IPC were much more highly enriched on metrizamide gradients: in the low-density fraction of 14.2 % metrizamide, in which 0.5-1.5 % of non-adherent cells were recovered, the IPC frequency was 20- to 130-fold increased as compared with unfractionated PBMC (range of results obtained in 11 experiments, table VI). To characterize the

Table IV. Influence of virus induction on positive selection of IPC with immunomagnetic beads.

No. exp.	mAb	Specificity	Immunomagnetic selection before virus-induction		Immunomagnetic selection after virus-induction	
			Percent cells	Frequency	Percent cells	Frequency
1	Control medium		11.3	7.0	4.2	11.0
	295/33	CD8	40.2	3.0	21.1	6.5
	74-12-4	CD4	26.5	17.0	21.8	27.0
	MSA3	SLA class II	15.8	3.3	14.1	24.0
2	Control medium		13.0	20.5	9.5	16.0
	74-12-4	CD4	40.1	65.6	23.3	148.5
	MSA3	SLA class II	33.9	41.0	29.7	120.0
	TH22A5	SLA class II	34.2	27.0	26.4	105.5

Non-adherent cells were treated with mAb and immunobeads before or after being incubated for 8 h with TGEV, at 2×10^6 cells/ml. The IPC frequencies ($\times 10^{-5}$) before immunomagnetic selection were 19.8 and 38.0 for exp. 1 and exp. 2, respectively.

Table V. IFN production in fractions from Percoll density gradients.

Cell fraction	Percent cells in fractions	IPC frequency ($\times 10^{-5}$)	IFN yield (units per IPC)
Unfractionated	100	48	1
40 % Percoll	1	125	0.8
45 % Percoll	5.7	450	3.3
50 % Percoll	20.1	30	0.7
> 55 % Percoll	73.2	0	0

Non-adherent cells were separated on a discontinuous Percoll gradient. Unfractionated cells were induced at 2×10^6/ml whereas Percoll gradient separated cells were induced at 2×10^5/ml. The number of cells recovered in the different fractions is expressed as the percentage of cells layered onto the gradients (unfractionated cells). IPC frequency was determined by ELISPOT assay. The IFN yield (units) per IPC was calculated from the amount of IFN in cell supernatants and the IPC number per culture (see "Materials and Methods"). Results from one out of three experiments are shown.

Table VI. IPC frequency in fractions from metrizamide density gradients.

Cell fraction	No. exp.	Percent cells in fractions	IPC frequency ($\times 10^{-5}$)
Unfractionated	11	100	31 ± 8.4
14.2 % metrizamide	11	1.2 ± 0.5	813.1 ± 493.9
15 % metrizamide	7	6.1 ± 4.2	335.4 ± 136.2
16 % metrizamide	5	16 ± 14.7	20.2 ± 13.7
17 % metrizamide	6	18.3 ± 8.1	1.5 ± 1.6
> 17 % metrizamide	6	61.4 ± 18.3	0

Non-adherent cells were separated on a discontinuous metrizamide gradient. Unfractionated cells were induced at 2×10^6/ml whereas metrizamide gradient separated cells were induced at 2×10^5/ml.

Table VII. Phenotype characterization of sequentially enriched subpopulations of blood cells.

mAb	Specificity	PBMC	Exp. 1 Non-adherent	Exp. 1 Metrizamide 14.2 %	Exp. 1 Fractions > 15 %	PBMC	Exp. 2 Non-adherent	Exp. 2 Metrizamide 14.2 %	Exp. 2 Fractions > 15 %
76-7-4	B cell	12	5	38	15	8	6	10	8
MSA4	CD2	46	33	51	41	59	54	61	54
72-12-4	CD4	32	19	21	16	30	31	43	22
295/33	CD8	27	36	50	15	36	42	35	32
TH22A5	SLA class II	ND	ND	ND	ND	17	23	32	13
MSA3	SLA class II	30	32	69	21	37	37	52	34
74-22-15	Macrophages/ granulocytes	27	28	41	18	21	14	38	17

Reactivity with mAb was determined by immunofluorescence. ND = not done.

enriched IPC population obtained on 14.2 % metrizamide gradients, indirect immunofluorescence tests were performed with anti-porcine leukocyte mAb. Table VII shows that low-density cell fractions were mainly enriched for TH22A5[+] or MSA3^{+} (SLA-class-II^{+}) cells and for 74-22-15^{+} cells (granulocytes/macrophages). To try to enrich IPC further, low-density cells isolated on 14.2 % metrizamide were also treated with different mAb: only a slight (less than 2-fold) increase in IPC frequency could be obtained when low-density cells were depleted of CD8^{+} cells (data not shown).

IFN yield per IPC in sequentially enriched cell populations

The mean amount of IFN produced by each IPC, as estimated from the amount of IFN in supernatants and the IPC number, was 3 units per IPC for PBMC incubated at 2×10^6/ml (data collected from 28 experiments; table I). When we compared the IFN yield per IPC among PBMC induced at different cell concentrations (from 1.2×10^5 to 4×10^6/ml), we observed that the IFN yield per IPC was not a constant value, as expected, but indeed increased with the increasing IPC concentration (fig. 1). When IPC were enriched in 45 % Percoll frac-

tions, we also observed an increase in IFN yield (table V). Finally, when all the data collected from cell fractionations on metrizamide (up to 11 experiments, table VI), were plotted, we found that IFN yield per IPC was more than 10-fold-increased with increasing IPC concentrations (fig. 2).
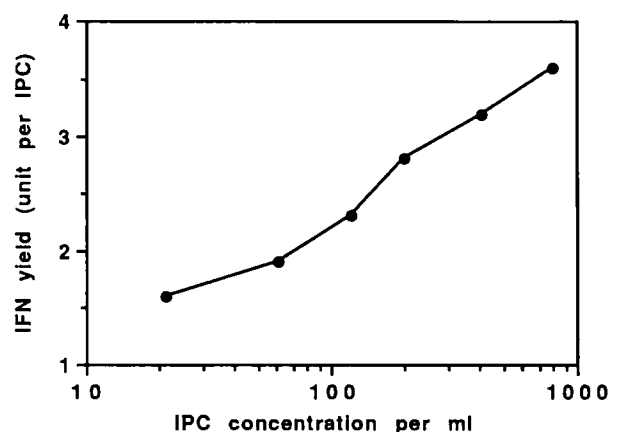

Fig. 1. Variations of IFN yields (units of IFN per IPC) as a function of PBMC concentrations.

Results are expressed as the means of 5 different experiments. For each PBMC concentration tested, the IPC concentration was monitored by ELISPOT and the IFN yield was estimated from the amount of IFN present in cell supernatants.
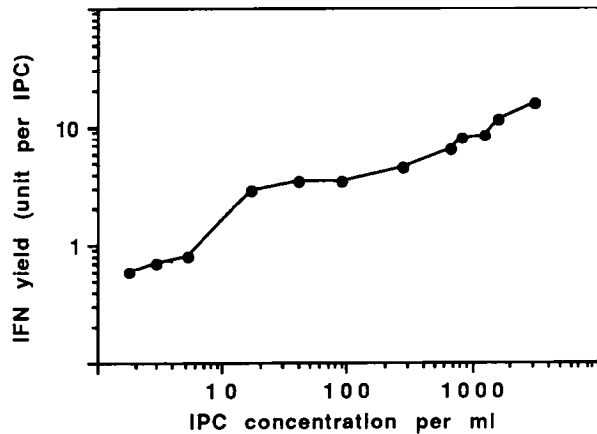
Fig. 2. Variations in IFN yield (units of IFN per IPC) as a function of concentrations of IPC enriched on metrizamide gradients.

Results are expressed as means of 5 to 11 different experiments. Non-adherent cells were fractionated on metrizamide gradients and each fraction (14.2 %, 15 %, 16 % and 17 %) was tested at 1, 2 and 4×10^5 cells/ml. IPC concentration and IFN yield per cell were calculated as described above for each cell preparation.

DISCUSSION

Our study indicates that the frequency of the porcine PBMC secreting IFNα in response to coronavirus TGEV, as monitored by ELISPOT assay, is extremely low (1 per 10^4) among PBMC (table I). This result is in agreement with other studies performed on HSV-induced human leukocytes. By several technical approaches, including limiting dilution analysis, RNA-RNA *in situ* hybridization, immunocytochemistry and ELISPOT assay, the frequency of human IPC in blood mononuclear cells is less than 1 per 10^3 (Gobl *et al.*, 1988; Feldman and Fitzgerald-Bocarsly, 1990; Cederblad and Alm, 1990). IPC, which circulate throughout the body and are induced to secrete IFNα following brief exposure to non-infectious viral structures (reviewed in Charley and Laude, 1992) are likely to contribute markedly to the early production of IFNα occurring in viral infections and thus deserve much attention. However, their very low frequency has greatly hampered their study. In the present report, we have analysed the effects of various cell fractionation procedures to enrich for coronavirus-induced porcine IPC. Depletion

of plastic-adherent and phagocytic cells resulted in a 3-fold IPC increase (table I), which indicates that, in contrast to the results of Gobl *et al.* (1988) demonstrating that monocytes are potent producers of IFNα in response to Sendai virus, TGEV-induced IFNα-secreting cells are non-adherent and non-phagocytic cells.

Specific cell fractionation experiments were then performed with anti-leukocyte mAb by complement-dependent lysis or by immunomagnetic bead selection. IPC frequency was greatly reduced following depletion of CD4+ cells performed before virus induction, whereas complement-mediated lysis of CD8+ cells slightly increased the IPC frequency. Fractionations of non-adherent cells by anti-SLA-class-II, anti-B or anti-CD2 mAb before virus induction did not affect IPC frequency (tables II and III). In contrast, when the immunomagnetic bead selection was performed after virus induction, IPC were positively selected by anti-CD4 as well as by anti-class-II mAb-coated beads (table IV).

These data indicate clearly that TGEV-induced IFNα-secreting cells, as monitored by ELISPOT, are characterized as non-adherent, non-phagocytic, non-T, non-B cells, expressing CD4 and SLA-class-II molecules, with the latter cell marker expressed by IPC following virus induction. In fact, virus-induced SLA-class-II expression on IPC could be the consequence of the effect of IFNα secreted by these cells. Such a cell phenotype is in agreement with the results obtained on HSV-induced human IPC (Sandberg *et al.*, 1989; Feldman and Fitzgerald-Bocarsly, 1990; Sandberg *et al.*, 1990; reviewed in Charley and Laude, 1992), which suggests that a similar and distinct cell population is responsible for IFNα production in several animal species following induction by different RNA or DNA viruses. Cell fractionations with mAb resulted in only a slight IPC enrichment: at the most, IPC were 4-fold-enriched by positive selection with anti-CD4 mAb-coated beads (exp. n° 2 in table IV). In contrast, cell fractionations on discontinuous density gradients allowed a much higher enrichment of IPC: in the low-density fractions from Percoll gradients and from metrizamide gradients (tables V and VI),

IPC were 10-30-fold enriched. The 14.2 % metrizamide fraction, which contained less than 1.5 % non-adherent cells, in some experiments contained IPC at a frequency up to 120 times higher than the starting cell population. This low-density cell population was mainly enriched in SLA-class-II$^+$ cells, in T cells and in cells reacting with a mAb specific for granulocytes and macrophages (table VII). In the human species, HSV- or CMV-induced IPC fractionated in a low-density cell population, enriched in HLA-DR$^+$ cells, but which appeared to be functionally distinct from dendritic cells (Feldman and Fitzgerald-Bocarsly, 1990; Chehimi *et al.*, 1989).

During the different cell fractionation procedures used in the present study to enrich in IPC, we observed that the estimated IFN yield per IPC was not a constant value for a given animal, as expected, but instead could vary with the IPC concentration. Thus, when non-adherent cells were incubated with TGEV at different cell concentrations, the IFN yield per cell increased with the IPC concentration (fig. 1). A similar observation was made when IPC were gradually enriched on density gradients (table V, fig. 2). The IFN yield per IPC was, in some experiments, 10 times higher after IPC enrichment (fig. 2).

Several mechanisms could account for increased IFN secretion by each IPC: accessory factors which enhance the IFNα response of IPC, such as IFNγ, IFNα, IL3 or GM-CSF (Cederblad and Alm, 1991), might be more highly produced in the cell fractions in which IPC were enriched. In fact, we could not detect IFNγ in the supernatants of TGEV-induced low-density cells (data not shown). Also, preliminary colony-stimulating factor (CSF) assays performed on porcine bone marrow semi-solid cultures indicated that there was no higher CSF activity in the supernatants of low-density cells (F. Hervatin, personal communication). It remains possible, therefore, that the increased IFN yield per IPC observed is a consequence of an enhancing, so called "priming", effect of IFNα upon its own production (Stewart *et al.*, 1971). Another possible explanation could be that cells having inhibitory influences on IFNα secretion were lost during the cells fractionation procedures.

The presently observed phenomenon may well have *in vivo* relevance during viral infections: thus, recruitment of inflammatory cells around initial sites of viral infection could locally increase the IPC concentration, thereby enhancing their capacity to secrete high amounts of IFNα, which could contribute to limiting the spread of virus infection.

L'enrichissement en leucocytes sanguins producteurs d'interféron après induction par un coronavirus accroît la quantité d'interféron produite par cellule

Les cellules mononucléées du sang périphérique du porc qui sécrètent l'IFNα après induction par le coronavirus de la gastroentérite transmissible, sont détectables par une technique immunoenzymatique sur filtre (ELISPOT). Ces cellules sont très peu fréquentes dans le sang mais ont pu être enrichies par déplétion des cellules adhérentes au plastique suivie d'une séparation cellulaire sur gradient de métrizamide. Les cellules sécrétrices d'IFNα ont été enrichies dans la fraction de faible densité des cellules non adhérentes. Des expériences de sélection des cellules à l'aide de billes magnétiques recouvertes d'anticorps ont montré que ces cellules étaient positivement sélectionnées par des anticorps anti-CD4 ou anti-SLA-classe-II mais non par des anticorps anti-CD2 ou -CD8. La production estimée d'IFN par cellule sécrétrice a augmenté dans les diverses situations où ces cellules se trouvaient être plus concentrées. Ces résultats suggèrent que les cellules sécrétrices d'IFNα représentent une sous-population cellulaire particulière des cellules sanguines, dont la capacité à produire l'IFN pourrait être accrue du fait de leur accumulation aux sites d'infection virale.

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