Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Res. Immunol.
1990, **141**, 141-151

CHARACTERIZATION OF BLOOD MONONUCLEAR CELLS PRODUCING IFNα FOLLOWING INDUCTION BY CORONAVIRUS-INFECTED CELLS (PORCINE TRANSMISSIBLE GASTROENTERITIS VIRUS)

B. Charley and **L. Lavenant**

Laboratoire de Virologie et d'Immunologie moléculaires,
INRA, 78350 Jouy-en-Josas (France)

SUMMARY

Porcine blood mononuclear cells (PBMC) were shown to produce interferon-α (IFNα) following incubation with cells infected by a coronavirus, transmissible gastroenteritis virus. Monoclonal antibodies (mAb) with specificities for leukocyte subsets and major histocompatibility complex (MHC) antigens were used to characterize IFNα producer cells. The production of IFNα was found to be a function of non-phagocytic, non-adherent, non-T, non-B, $CD4^+$ (and to a lesser extent $CD8^+$) MHC-class-II-positive cells. Furthermore, addition of anti-MHC (class II) mAb during PBMC incubation with virus-infected cells reduced IFN yields, suggesting that masking of these surface antigens alters PBMC responsiveness to IFN induction.

KEY-WORDS: Interferon-α, Leukocyte, MHC, Coronavirus; Transmissible gastroenteritis virus, mAb, PBMC.

INTRODUCTION

Peripheral blood leukocytes have been shown to produce interferon alpha (IFNα) upon induction by viruses, bacterial products or tumour cells (Dianzani and Capobianchi, 1987). In contrast to IFN beta, for which the critical inducing factor appears to be viral RNA, a different mechanism seems to be involved in induction of IFNα. Thus, inactivated virus particles, virus-infected cells, mycoplasmas and cell membranes are capable of inducing IFNα (Lebon *et al.*, 1982; Hughes and Blalock, 1983; Gobl *et al.*, 1988; Capobianchi *et al.*., 1987), which suggests that membrane interactions between the inducer structure and the leukocyte membrane is sufficient to trigger IFNα produc-

tion. In the case of transmissible gastroenteritis virus (TGEV), a coronavirus which induces acute diarrhœa and intense IFN synthesis in newborn piglets (La Bonnardiere and Laude, 1981), we have previously shown that early IFNα production could result from exposure of non-immune pig lymphocytes to virus-infected cells. Moreover, experiments in which IFNα production was inhibited by monoclonal antibodies (mAb) directed at some epitopes of the TGEV glycoprotein E1 suggested that a defined domain of this transmembrane viral protein played a key role in the IFNα induction process (Charley and Laude, 1988).

The nature of IFNα producer cells (IPC) is not clear, since several cell types appear to be involved depending upon the inducer or the induction protocol (Dianzani and Capobianchi, 1987). Thus, among non-adherent human mononuclear cells, "nuli" (non-T, non-B) lymphocytes and large granular lymphocytes (LGL) were shown to produce IFNα upon exposure to herpes, influenza or dengue virus (Peter *et al.*, 1980; Kirchner *et al.*, 1979; Lebon *et al.*, 1982; Djeu *et al.*, 1982; Kurane *et al.*, 1986). More recent reports have indicated that a common phenotypic feature for murine and human IPC in response to different stimuli is their surface expression of MHC class II molecules (Abb *et al.*, 1983; Reiss *et al.*, 1984; Perussia *et al.*, 1985; Kurane and Ennis, 1987; Hughes and Blalock, 1983; Capobianchi *et al.*, 1987; Oh *et al.*, 1987; Fitzgerald-Bocarsky *et al.*, 1988; Sandberg *et al.*, 1989a).

In the present report, we analysed the nature of lymphocyte subpopulation(s) responsive to IFNα induction by TGEV-infected cell monolayers by using cell-depletion experiments with mAb plus complement. In addition, IFN induction-blocking experiments conducted with anti-MHC (SLA, swine leukocyte antigens) class II mAb without complement suggest a functional role for these membrane molecules in the IFN induction process.

MATERIALS AND METHODS

PBMC.

Porcine peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood collected from 2- to 4-month old animals. Phagocytic cells were depleted by carbonyl iron ingestion (Salmon, 1979) before PBMC isolation by Ficoll density centrifugation on MSL (density 1.077, Eurobio, Paris). PBMC were suspended in RPMI-1640 medium supplemented with 10 % fœtal calf serum.

IFN = interferon.
IPC = IFNα producer cell.
LGL = large granular lymphocyte.
mAb = monoclonal antibody.
MHC = major histocompatibility complex.
PBMC = peripheral blood mononuclear cell.
SLA = swine leukocyte antigen.
TGEV = transmissible gastroenteritis virus.

mAb.

Anti-PBMC mAb MSA4 (anti-CD2), 76-7-4 (anti-B), 74-12-4 (anti-CD4) were kindly provided by J. Lunney (USDA, Beltsville, MD, USA); 295/33 (anti-CD8) mAb was kindly provided by U. Koszinowski (Tübingen, FRG). mAb 2-2-13 (anti-SLA class I) and MSA3 (anti-SLA class II) were provided by J. Lunney. mAb TH22A5 (anti-SLA class II) was purchased from VMRD (Pullman, WA, USA). Other anti-SLA class II mAb (TH14B, H42A and TH81A) were kindly provided by W. Davis (Pullman, WA, USA) (table I). These antibodies were used for cell-depletion experiments as ascitic fluids, excepts for MSA3 which was used as hybridoma cell supernatant.

Treatment of PBMC with mAb and complement.

PBMC were incubated for 30 min with mAb and complement at 37°C as described previously (Charley *et al.*, 1987): briefly, one-month old rabbit serum served as a source of complement at a final dilution of 1/9 and mAb were used at a final dilution of 1/300. The percentage of dead cells was determined by trypan blue dye exclusion and cells were resuspended in the initial volume, *i.e.* without readjustment of the viable cell concentration, before being used in the assays.

IFN induction.

PBMC were induced to produce IFNα by overnight incubation on TGEV-induced, glutaraldehyde-fixed cell monolayers as described previously (Charley and Laude, 1988): briefly, pig kidney cells were plated in 96-well microplates, infected by the coronavirus TGEV for 18 h, then fixed with 0.25 % glutaraldehyde (1 h at 4°C) and stored with 3 % glycine. Monolayers were washed before addition of PBMC (100 µl/well at 5×10^6/ml). Supernatants were collected after 18 h of incubation at 37°C and assayed for IFN activity.

IFN blocking experiments with anti-SLA class II mAb.

Various dilutions of dialysed mAb preparations, as indicated in "Results", were added during overnight incubation of PBMC with TGEV-infected monolayers or with virus particles. Alternatively, PBMC were pretreated with various amounts of dialysed

TABLE I. — **mAb used for this study.**

mAb	Specificity	Isotype	Origin
MSA4	CD2, pan-T	IgG2a	Hammerberg and Schurig, 1986
76-7-4	CD1, B cell	IgG2a	Pescovitz *et al.*, 1984
295/33	CD8, cytotoxic T cells	IgG2a	Jonjic and Koszinowski, 1984
74-12-4	CD4, helper T cells	IgG2b	Pescovitz *et al.*, 1984
2-2-13	SLA class I	–	Davis *et al.*, 1984
MSA3	SLA class II	IgG2b	Hammerberg and Schurig, 1986
TH22A5	SLA class II	IgG2a	VMRD, Inc., Pullman WA
TH14B	SLA class II	IgG2a	Davis *et al.*, 1984
H42A	SLA class II	IgG2a	Davis *et al.*, 1984
TH81A5	SLA class II	IgG2a	Davis *et al.*, 1984

mAb preparations for 60 min at 37°C, washed to remove unbound material, resuspended at 5×10^6/ml and incubated overnight on TGEV-infected monolayers. Supernatants were assayed for IFN.

IFN bioassay.

Log_3 dilutions of PBMC supernatants were assayed for IFN on bovine MDBK cells using vesicular stomatitis virus as a challenge (La Bonnardière and Laude, 1981). A 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 is equivalent to 1IU of human IFN.

RESULTS

1) Effect of antibody and complement treatment of IFNα producer cells.

We have previously shown that phagocyte-depleted porcine PBMC could secrete IFNα following incubation on TGEV-infected glutaraldehyde-fixed cell monolayers (Charley and Laude, 1988). Antibody plus complement depletion experiments were conducted to characterize the IPC nature. When PBMC were pretreated with rabbit serum as a source of complement, they produced high amounts of IFN (4 800 ± 3 000 U/ml in 10 different experiments). Table II shows the effect of treatment with various anti-lymphocyte mAb and complement on IFN production. Since IFN assays are performed on log_3 dilutions of PBMC supernatants, any reduction lower than 33 % was considered as negligible. In fact, pretreatment of PBMC with anti-T or anti-B cell mAb plus complement did not alter IFNα production: IFN titres obtained were respectively equal to 45 and 71 % of titres obtained with complement-treated PBMC (table II). In contrast, pretreatment with 245/33 (anti-CD8) mAb and,

TABLE II. — **Effect of PBMC pretreatment with antilymphocyte mAb and complement on IFNα production after induction with TGEV-infected glutaraldehyde-fixed cell monolayers.**

mAb	Specificity	No.	% Viable cells	IFN production (% of control)
MSA4	T cells	16	58.2 ± 4	45.3 ± 8.7
76-7-4	B cells	12	92.6 ± 1.5	71.5 ± 9.8
74-12-4	CD4	4	67.7 ± 1.7	5.4 ± 1.9
295/33	CD8	13	65.8 ± 3.6	17.7 ± 2.7

No. = number of experiments.
Viable cells = 100 % − (% dead cells after effect of mAb + complement − % dead cells with complement alone).
IFN production is expressed as % of IFN produced by complement-pretreated PBMC.

TABLE III. — **Effect of pretreatment of porcine PBMC by anti-SLA mAb and complement on IFNα production after induction with TGEV-infected glutaraldehyde-fixed cell monolayers.**

mAb	Specificity	No.	% Viable cells	IFN production (% of control)
2-2-13	SLA class I	2	10 ±2.8	< 1
TH22A5	SLA class II	3	76.3±2.5	18 ± 7.5
MSA3	SLA class II	4	86.3±2.2	50.2±16.5

Same legends as in table II.

to a greater extent, with 74-12-4 (anti-CD4) mAb produced 80 to 95 % reduction in IFN yield (table II). This series of experiments therefore indicated that the majority of porcine IPC induced by TGEV-infected cells are non-T, non-B, $CD4^+$ (and to a lesser extent $CD8^+$) cells.

Similar types of experiments were conducted with mAb directed against MHC (SLA), class I and class II products. Table III shows that pretreatment by anti-SLA class I mAb and complement, which destroyed almost all PBMC, completely abolished IFN production. Pretreatment by anti-SLA class II mAb TH22A5 plus complement markedly lowered IFN production, along with the lysis of 14 % PBMC. These experiments therefore indicated that porcine IPC were largely SLA-class-II-positive cells.

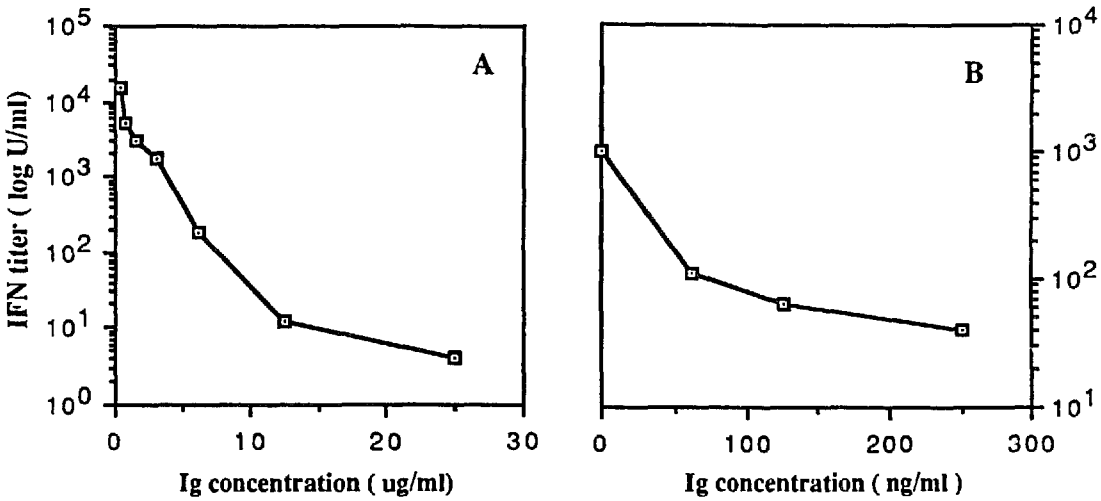

FIG. 1. — *Monoclonal antibodies to SLA class II antigens block IFNα induction.*

Various concentrations of TH22A5 ascites (A) or MSA3 hybridoma culture supernatant (B) were added to PBMC (5×10^6/ml) incubated overnight with TGEV (at a concentration of 105 plaque-forming units/ml). IFN activity was assayed in culture supernatants.

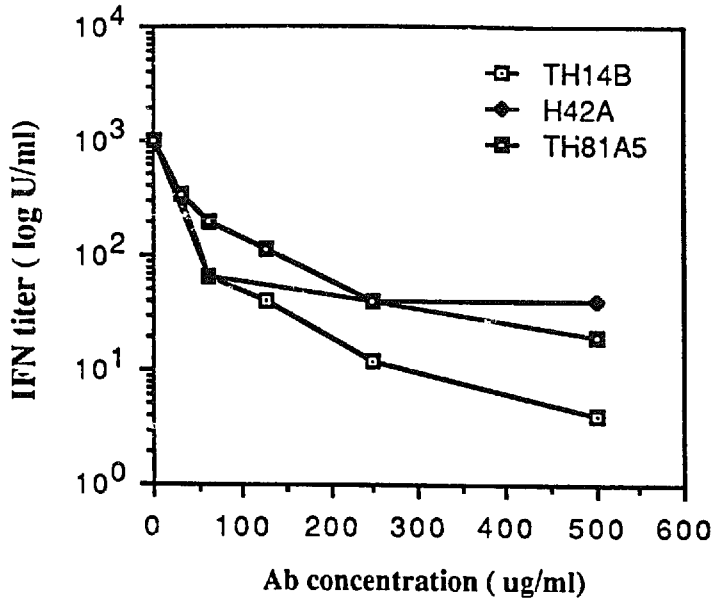

FIG. 2. — *Pretreatment of PBMC with anti-SLA class II mAb reduces IFNα yield.*

PBMC were pretreated with various concentrations of anti-SLA class II ascites fluids (60 min. at 37°C), washed and incubated overnight on TGEV-infected glutaraldehyde-fixed cell monolayers. IFN activity was assayed in cell supernatants.

2) Effect of antibody in the absence of complement on IFNα production.

During cell-depletion experiments, controls performed in the absence of complement indicated that most mAb used had no direct inhibitory effects on IFN production. However, two preparations of anti-SLA class II mAb (TH22A5 and MSA3) could directly block IFN induction when added during co-incubation of PBMC with TGEV-infected fixed monolayers or TGE alone (5 independent experiments). Thus, TH22A5 mAb used as an ascitic fluid could inhibit up to 99.9 % IFN production at a final Ig concentration of 12 µg/ml (fig. 1a). MSA3 hybridoma culture supernatant blocked up to 99 % IFN production at a final Ig concentration of 0.25 µg/ml (fig. 1b). Three other ascitic fluids, directed at SLA class II, also showed dose-dependent inhibition of IFN production (fig. 2). In addition, the latter blocking experiment was achieved by pretreatment of PBMC with mAb, followed by washing of cells before induction on TGEV-infected fixed-cell monolayers, which argues for a direct effect of mAb on PBMC as opposed to an antibody effect on infected cell monolayers. In addition, such inhibiting effects could not be related to toxic effects of mAb on PBMC (not shown). IFN induction by other viruses such as Newcastle disease virus (NDV), Sendai virus and types A and B myxoviruses was also blocked by co-incubation of PBMC with TH22A5 mAb (not shown).

DISCUSSION

We have previously shown that phagocyte-depleted PBMC incubated with TGEV-infected cells are rapidly induced to secrete IFNα (Charley and Laude, 1988). This IFNα induction seems to result from membrane interactions between PBMC and a defined domain of the viral glycoprotein E1 expressed on the surface of infected cells (Charley and Laude, 1988). In the present paper, cell-depletion experiments by mAb and complement-dependent lysis indicate that porcine IFNα producer cells (IPC) are mostly $MSA4^-$ (CD2 or pan-T)$^-$ and 76-7-4^- (B) cells, whereas depletion of $CD4^+$ cells, and to a lesser extent $CD8^+$ cells reduced IFN yields by 95 and 82 %, respectively (table II). In addition, IPC are also SLA class I^+ and class II^+ cells. Furthermore, the addition of anti-SLA class II mAb reduces IFNα production.

Our present data on the nature of porcine IPC are in agreement with several reports about the characterization of human IPC. Thus, following induction by different viruses including herpes virus, influenza virus, dengue virus or cytomegalovirus, as well as by mycoplasma membranes, human mononuclear leukocytes producing IFNα were described as non-adherent, non-phagocytic, non-T,non-B cells (Trinchieri *et al.*, 1978; Kirchner *et al.*, 1979; Peter *et al.*, 1980; Lebon *et al.*, 1982; Abb *et al.*, 1983; Djeu *et al.*, 1982; Perussia *et al.*, 1985; Kurane *et al.*, 1986). Human IPC were generally shown to lack natural killer function (Lebon *et al.*, 1982; Abb *et al.*, 1983; Fitzgerald-Bocarsly *et al.*, 1988), but to express MHC class II antigens (Abb *et al.*, 1983; Perussia *et al.*, 1985; Capobianchi *et al.*, 1987; Oh *et al.*, 1987; Fitzgerald-Bocarsly *et al.*, 1988). A recent report using combined immunocytochemistry and *in situ* RNA-RNA hybridization on human PBMC stimulated by HSV-infected cells clearly showed that IPC lacked antigens typical of T and B lymphocytes, but expressed HLA-class II antigens (Sandberg *et al.*, 1989a). The same laboratory observed that IPC also expressed CD4 antigens (Sandberg *et al.*, 1989b). The expression of MHC class II antigens led to the hypothesis that human IPC could be dendritic cells (Fitzgerald-Bocarsly *et al.*, 1988). However, HLA-DR^+ cells could recently be divided into two separate subsets: a loosely adherent population meeting the functional criteria of dendritic cells but distinct from IPC which were non-adherent (Chehimi *et al.*, 1989). Our data therefore indicate that porcine IFNα-producing cells in response to TGEV-infected cells have the same properties as human IPC: porcine IPC are non-phagocytic, non-adherent (Salmon *et al.*, 1989), non-T,non-B, MHC-class-II-positive and $CD4^+$ cells. Preliminary experiments using DNA-RNA *in situ* hybridization suggested that porcine IFNα-mRNA-containing cells were infrequent (around $1/10^4$ PBMC; Buseyne and Charley, unpublished observations) as already described for human IPC (Gobl *et al.*, 1988). Interestingly, one might hypothesize that a defined, albeit infrequent cell population exhibiting unusual phenotypic features could produce IFNα in response to a wide range of IFN inducers. In order to further analyse these

cells and their mode of activation, it will be necessary to use positive selection procedures such as cell sorting (Sandberg *et al.*, 1989b).

Blocking experiments conducted with anti-SLA-class II MSA3 mAb used as hybridoma culture supernatant revealed a reduction in IFN yield of more than 95 % (fig. 1). Comparable inhibition was obtained with 4 other anti-SLA class II mAb used as ascitic fluids. This inhibition is not observed when virus-infected cells are pretreated with mAb, then washed before PBMC are added. However, when PBMC are pretreated with mAb, then washed before induction, IFN yield is still reduced, which argues for a direct effect of anti-SLA class II antibodies on PBMC. In addition, PBMC are not lysed by such treatments. Therefore, these results suggest that masking of MHC class II antigens on the PBMC membrane reduces their responsiveness to IFN induction. This anti-SLA class-II-Ab-mediated blockage is observed when PBMC are induced by TGEV, Sendai, NDV or influenza virus. A similar observation was reported by Capobianchi *et al.* (1987): pretreatment of human PBMC by anti-HLA-DR antibody reduced IFNα yield after induction with mycoplasma membranes. Similarily, Ia antigens were shown to bind cell surface glycoproteins responsible for IFN induction (Hughes *et al.*, 1986). Therefore, our findings suggest that, in addition to mycoplasmas and cell surface glycoproteins, viruses could also interact with MHC class II antigens to induce IFNα synthesis.

The fact that masking of MHC class II could reduce PBMC responsiveness to several IFN inducers suggests that these surface antigens are not virus-specific receptors on lymphoid cells. In fact, anti-SLA class II mAb did not block TGEV replication in susceptible pig kidney cells (data not shown). The actual functional role of MHC class II molecules in the IFNα induction process remains to be clarified: they could represent broadly reactive recognition structures able to bind different IFN-inducing components (as suggested by Hughes *et al.*, 1986). Alternatively, MHC class II molecules could be involved in post-recognition events, such as internalization or recycling of membrane structures, which seem to precede activation of IFNα synthesis (Lebon, 1985). Experiments are in progress to further define the nature of virus lymphocyte interactions leading to IFNα production.

RÉSUMÉ

CARACTÉRISATION DES CELLULES SANGUINES MONONUCLÉES PRODUISANT L'INTERFÉRON ALPHA APRÈS INDUCTION PAR DES CELLULES INFECTÉES PAR LE CORONAVIRUS PORCIN GET

Des cellules sanguines mononuclées du porc produisent de l'interféron α (IFNα) à la suite de leur incubation avec des cellules infectées par le coronavirus GET (gastroentérite transmissible). Les cellules productrices d'interféron ont été caractérisées à l'aide d'anticorps monoclonaux (AcM) spécifiques des sous-populations leucocytaires et des antigènes du complexe majeur d'histocompatibilité (CMH). Les cellules productrices d'IFNα sont des cellules non phagocytaires, non adhérentes, ni T, ni

B, CD4+ (pour une moindre part CD8+) et CMH-classe-II+. De plus, l'addition d'AcM dirigés contre les antigènes de classe II du CMH, au mélange d'incubation cellules mononuclées plus cellules infectées par le virus GET, réduit la production d'IFNα, ce qui suggère que le masquage de ces antigènes de surface modifie la capacité des cellules mononuclées à répondre aux signaux inducteurs d'IFNα.

MOTS-CLÉS: Interféron alpha, Lymphocyte, CMH, Coronavirus; Anticorps monoclonaux, Virus de la Gastroentérite transmissible du porc.

ACKNOWLEDGEMENTS

We are grateful to Dr. J. Lunney (Beltsville, MD, USA), Dr. U. Koszinowski (Tübingen, FRG) and Dr. W. Davis (Pullman, WA, USA), who kindly provided anti-porcine lymphocyte subsets and anti-SLA monoclonal antibodies. We also thank Dr. F. Blecha (Manhattan, KS, USA) for revising the manuscript.

REFERENCES

ABB, J., ABB, H. & DEINHARDT, F. (1983), Phenotype of human alpha interferon-producing leucocytes identified by monoclonal antibodies. *Clin. exp. Immunol.*, **52**, 179-184.

CAPOBIANCHI, M.R., LORINO, G., LUN, M.T., MANCINI, C., DI MARCO, P. & DIANZANI, F. (1987), Membrane interactions involved in the induction of interferon-alpha by *Mycoplasma pneumoniae*. *Antiviral Res.*, **8**, 115-124.

CHARLEY, B., LAUDE, H. & LA BONNARDIÈRE, C. (1987), Inhibition of transmissible gastroenteritis coronavirus (TGEV) multiplication *in vitro* by non-immune lymphocytes. *Ann. Inst. Pasteur/Virol.*, **138**, 183-194.

CHARLEY, B. & LAUDE, H. (1988). Induction of alpha interferon by transmissible gastroenteritis coronavirus: role of transmembrane glycoprotein E1. *J. Virol.*, **62**, 8-11.

CHEHIMI, J., STARR, S.E., KAWASHIMA, H., MILLER, D.S., TRINCHIERI, G., PERUSSIA, B. & BANDYOPADHYAY, S. (1989), Dendritic cells and IFN-α-producing cells are two functionally distinct non-B,non-monocytic HLA-DR+ cell subsets in human peripheral blood. *Immunology*, **68**, 486-490.

DAVIS, W.C., MARUSIC, S., LEWIN, H.A., SPLITTER, G.A., PERRYMAN, L.E., MCGUIRE, T.C. & GORHAM, J.R. (1987) The development and analysis of species-specific and cross-reactive monoclonal antibodies to leukocyte differentiation antigens and antigens of the major histocompatibility complex for use in the study of the immune system in cattle and other species. *Vet. Immunol. Immunopathol.*, **15**, 337-376.

DIANZANI, F. & CAPOBIANCHI, M.R. (1987). Mechanism of induction of alpha interferon, *in* "The interferon system. A current review to 1987" (Baron, S., Dianzani, F., Stanton, J., Fleischmann, W.R.) (p. 21-30). University of Texas Medical Branch Series in Biomedical Science.

DJEU, J.Y., STOCKS, N., ZOON, K., STANTON, G.J., TIMONEN, T. & HERBERMAN, R.B. (1982), Positive self regulation of cytotoxicity in human natural killer cells by production of interferon upon exposure to influenza and herpes viruses. *J. exp. Med.*, **156**, 1222-1234.

FITZGERALD-BOCARSLY, P., FELDMAN, M., MENDELSOHN, M., CURL, S. & LOPEZ, C. (1988), Human mononuclear cells which produce interferon-alpha during NK (HSV-FS) assays are HLA-DR-positive cells distinct from cytolytic naturel killer effectors. *J. Leuk. Biol.*, **43**, 323-334.

GOBL, A.E., FUNA, K. & ALM, G.V. (1988), Different induction patterns of mRNA for IFN-α and -ß in human mononuclear leukocytes after *in vitro* stimulation with herpes simplex virus-infected fibroblasts and Sendai virus. *J. Immunol.*, **140**, 3605-3609.

HAMMERBERG, C. & SCHURIG, G.G. (1986), Characterization of monoclonal antibodies directed against swine leukocytes. *Vet. Immunol. Immunopathol.*, **11**, 107-121.

HUGHES, T.K. & BLALOCK, J.E. (1983), Ia antigen: a murine B lymphocyte receptor for transformed cell induction of interferon-α/ß. *J. Immunol.*, **131**, 1895-1899.

HUGHES, T.K., SMITH, E.M. & BLALOCK, J.E. (1986), Interferon-inducing transformed cell surface glycoproteins: purification by Ia antigen affinity chromatography. *Proc. Soc. exp. Biol.* (N.Y.), **182**, 564-567.

JONJIC, S. & KOSZINOWSKI, U.H. (1984), Monoclonal antibodies reactive with swine lymphocytes.-I. Antibodies to membrane structures that define the cytolytic T lymphocyte subset in the swine. *J. Immunol.*, **133**, 647-652.

KIRCHNER, H., PETER, H.H., HIRT, H.M., ZAWATZKY, R., DALÜGGE, H. & BRADSTREET, P. (1979), Studies of the producer cell of interferon in human lymphocyte cultures. *Immunobiol.*, **156**, 65-75.

KURANE, I. & ENNIS, F.A. (1987), Induction of interferon-α from human lymphocytes by autologous, Dengue virus-infected monocytes. *J. exp. Med.*, **166**, 999-1010.

KURANE, I., MEAGER, A. & ENNIS, F.A. (1986), Induction of interferon alpha and gamma from human lymphocytes by Dengue virus-infected cells. *J. gen. Virol.*, **67**, 1653-1661.

LA BONNARDIÈRE, C. & LAUDE, H. (1981), High interferon titer in newborn pig intestine during experimentally induced viral enteritis. *Infect. Immun.*, **32**, 28-31.

LEBON, P. (1985), Inhibition of Herpes Simplex virus type-1-induced interferon synthesis by monoclonal antibodies against viral glycoprotein D and by lysosomotropic drugs. *J. gen. Virol.*, **66**, 2781-2786.

LEBON, P., BERNARD, A. & BOUMSELL, L. (1982), Human lymphocytes involved in α-interferon production can be identified by monoclonal antibodies directed against cell surface antigens. *C.R. Acad. Sci.* (Paris), **295**, 79-82.

OH, S.H., BANDYOPADHYAY, S., MILLER, D.S. & STARR, S.E. (1987), Cooperation between CD16 (Leu-11b)$^+$ NK cells and HLA-DR$^+$ cells in natural killing of herpesvirus-infected fibroblasts. *J. Immunol.*, **139**, 2799-2802.

PERUSSIA, B., FANNING, V. & TRINCHIERI, G. (1985), A leukocyte subset bearing HLA-DR antigens is responsible for *in vitro* interferon production to viruses. *Nat. Immun. Cell Growth Reguls.*, **4**, 120-137.

PESCOVITZ, M.D., LUNNEY, J.K. & SACHS, D.H. (1984), Preparation and characterization of monoclonal antibodies reactive with porcine PBL. *J. Immunol.*, **133**, 368-375.

PETER, H.J., DALLIGGE, H., ZAWATZKY, R., EULER, S., LEIBOLD, W. & KIRCHNER, H. (1980), Human peripheral null lymphocytes.-II. Producers of type 1 interferon upon stimulation with tumor cells, herpes simplex virus and *Corynebacterium parvum*. *Europ. J. Immunol.*, **10**, 547-555.

REISS, C.S., LIU, L.L.M. & MOWSHOWITZ, S.L. (1984), Interferon production by cultured murine splenocytes in response to influenza virus-infected cells. *J. Interferon Res.*, **4**, 81-89.

SALMON, H. (1979), Surface markers of porcine lymphocytes and distribution in various lymphoïd organs. *Int. Arch. Allergy.*, **60**, 262-274.

SALMON, H., CHARLEY, B., LA BONNARDIÈRE, C., OLIVIER, M., KELLEY, K. & PARAF, A. (1989), Natural killer (Nk) activity and interferon (IFN) production by a fraction of spleen and blood lymphocytes in swine. *Vet. Immunol. Immunopathol.*, **23**, 113-128.

SANDBERG, K., GOBL, A.E., FUNA, K. & ALM, G.V. (1989a), Characterization of the blood mononuclear leucocytes producing alpha interferon after stimulation with herpes simplex virus *in vitro*, by means of combined immunohistochemical staining and *in situ* RNA-RNA hybridization. *Scand. J. Immunol.*, **29**, 651-658.

SANDBERG, K., MATSSON, P. & ALM, G.V. (1989b). CD4 antigen are expressed on the interferon-α-producing cells induced by herpes simplex-infected cells. *J. Interferon Res.*, **9**, suppl. 2, (Abstract n° A7-2).

TRINCHIERI, G., SANTOLI, D., DEE, R.R. & KNOWLES, B.B. (1978). Antiviral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Identification of the antiviral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J. exp. Med.*, **147**, 1299-1313.