



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.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

THE ROLE OF INTERFERON IN SPONTANEOUS CELL-MEDIATED CYTOTOXICITY IN PIGS

A. CEPICA^{1,2} and J.B. DERBYSHIRE

Department of Veterinary Microbiology and Immunology, University of Guelph, Guelph, Ontario N1G 2W1 (Canada)

(Accepted for publication 11 June 1985)

ABSTRACT

Cepica, A. and Derbyshire, J.B., 1986. The role of interferon in spontaneous cell-mediated cytotoxicity in pigs. *Vet. Microbiol.*, 11: 69–77.

Specific release of ⁵¹Cr and the production of interferon (IFN) increased in parallel in a spontaneous cell-mediated cytotoxicity (SCMC) assay in which uninfected PK-15 cells or PK-15 cells persistently infected with transmissible gastroenteritis virus (PK-15-TGE cells) were used as targets, and peripheral blood lymphocytes (PBL) from a young adult pig were used as effector cells. Higher levels of both specific ⁵¹Cr release and IFN were obtained in the assays containing PK-15-TGE cells. Co-cultivation of PBL from newborn piglets with PK-15-TGE cells yielded similar levels of IFN to those produced by co-cultivation of adult PBL and PK-15-TGE cells, but lower levels of IFN were produced by co-cultivation with uninfected PK-15 cells. Pretreatment of adult PBL with IFN augmented their SCMC effector activity for both PK-15 and PK-15-TGE cells in the ⁵¹Cr release assay. Pretreatment of the PK-15-TGE target cells with IFN did not affect their release of ⁵¹Cr in the SCMC reaction, while IFN pretreatment of PK-15 targets protected them against SCMC.

In a single cell cytotoxicity assay the effects of IFN pretreatment on the effector adult PBL and on the PK-15 and PK-15-TGE target cells were confirmed, and SCMC incompetent PBL from neonatal piglets were rendered cytotoxic by pretreatment with IFN. PBL from newborn piglets bound to either target cell with the same frequency as PBL from SCMC competent adult pigs, and IFN pretreatment of either effector or target cells had no effect on target-binding frequency.

INTRODUCTION

In an earlier paper (Cepica and Derbyshire, 1983) we described spontaneous cell-mediated cytotoxicity (SCMC) against target cells infected with transmissible gastroenteritis virus (TGEV), a porcine enteric coronavirus,

¹ Present Address: Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, P.E.I., Canada, C1A 4P3.

² To whom reprint requests should be addressed.

mediated by porcine peripheral blood lymphocytes (PBL) and intraepithelial lymphocytes (IEL). SCMC by porcine lymphocytes against human myeloid leukemia (K562) cells (Kim et al., 1980; Norley and Wardley, 1983), against IB.RS.2 cells and GBK cells infected with pseudorabies virus has also been described. We have shown that PBL and IEL from neonatal piglets are incapable of acting as effector cells in SCMC against TGEV-infected targets (Cepica and Derbyshire, 1984a), as are neonatal PBL against K562 cells (Kim et al., 1980). The adoptive transfer of PBL from an adult pig to inbred neonatal recipients established SCMC effector activity in the PBL and IEL of the latter (Cepica and Derbyshire, 1984b), and challenge of the recipient piglets with TGEV indicated that the adoptive transfer of PBL increased their resistance to infection, suggesting that SCMC may be an important defence mechanism against this enteric virus. This finding raised the possibility of activating SCMC effector activity in the newborn, thereby increasing resistance to neonatal viral infection.

Augmentation of SCMC effector activity by interferon (IFN) is well established in the mouse (Gidlund et al., 1978; Djeu et al., 1979) and in man (Trinchieri et al., 1978). In the pig it has been shown that SCMC against K562 cells can be enhanced by incubation of effector lymphocytes from young adults with either porcine or human IFN (Charley et al., 1983). Lysis of IB.RS.2 cells, both uninfected and infected with pseudorabies virus, by PBL, was also enhanced by human IFN (Martin and Wardley, 1984), but studies on the role of IFN in SCMC by porcine lymphocytes against TGEV-infected targets, and of the effects of IFN on SCMC effector lymphocytes from neonatal piglets are lacking.

The objective of the present study was to investigate the role of IFN in SCMC against TGEV-infected target cells. The kinetics of cytotoxicity and IFN production in the SCMC chromium (^{51}Cr) release assay are described, and the effects of pretreatment with porcine IFN of the effector and target cells in both the SCMC ^{51}Cr release assay and in a single cell cytotoxicity assay for SCMC are reported. The latter assay was also used to determine the ability of SCMC incompetent PBL from neonatal pigs to bind to target cells, and to acquire cytotoxic activity after treatment with IFN.

MATERIALS AND METHODS

SCMC chromium release assay

The target cells (Cepica and Derbyshire, 1983) were PK-15 cells or PK-15 cells persistently infected with TGEV (PK-15-TGE cells). The effector cells were PBL from young adult pigs lacking TGEV neutralizing antibodies, isolated and purified by centrifugation over Ficoll-Hypaque, with removal of monocytes by plastic adherence, as described by Cepica and Derbyshire (1984a). The assays were performed as described previously (Cepica and

Derbyshire, 1983), with an effector:target cell ratio of 50:1 and incubation for 16 h. Appropriate controls were included as before, and all determinations were made in triplicate except for the controls for spontaneous and total ^{51}Cr release, for which six wells were used. The results are expressed as % specific ^{51}Cr release, computed as $100 \times [(\text{mean sample release} - \text{mean spontaneous release}) / (\text{mean total release} - \text{mean spontaneous release})]$. Each release was measured in counts per minute.

Co-cultivation of target and effector cells

PK-15 and PK-15-TGE cells were grown to confluency in Falcon 25 cm² tissue culture flasks. The media were discarded, the monolayers rinsed twice with phosphate buffered saline, and 10 ml of complete RPMI-1640 medium (Cepica and Derbyshire, 1983) containing 2.6×10^7 PBL isolated from young adults or neonatal pigs were added to each flask. Controls comprised PK-15 and PK-15-TGE cells without PBL, and PBL without target cells. The cultures were incubated for 16 h at 37°C, after which the media were collected and centrifuged at $200 \times g$ for 15 min. The supernatants were sterilized by ultraviolet irradiation by exposure in a 1.0 mm layer to a General Electric G15T8 15 Watt ultraviolet lamp at a distance of 15 cm for 15 min and stored at -70°C prior to assay for IFN activity.

Assay of interferon

Supernatants from SCMC ^{51}Cr release assays and supernatants from co-cultivated target and effector cells were assayed for antiviral activity against vesicular stomatitis virus. Monolayers of stable swine testis (SST) cells (McClurkin and Norman, 1966) were tested with dilutions of the appropriate supernatants for 20 h and then challenged with approximately 20 plaque-forming units of vesicular stomatitis virus. Antiviral units of IFN activity are expressed as the reciprocal of the highest dilution of supernatant that inhibited the formation of $\geq 50\%$ of the plaques.

Single cell cytotoxicity assay

The test was based on that described by Grimm and Bonavida (1979). Briefly, trypsinized PK-15 or PK-15-TGE cells (2.5×10^6) were mixed with equal numbers of adult or neonatal PBL. The mixture was centrifuged for 5 min at $100 \times g$, and the pelleted cells incubated for 5 min at 37°C to allow binding of lymphocytes to target cells. The cells were resuspended, 2.0 ml of 1% agarose were added, and 1.0 ml was spread in a Falcon tissue culture dish. Additional layers of 2.0 ml of 1% agarose, and 1.0 ml of complete RPMI-1640 were added. After incubation for 16 h at 37°C in 5% CO₂, the fluid medium was removed, 0.1% trypan blue was added and incubation continued for 15 min. The stain was then replaced with 0.5%

formaldehyde. The number of target-binding lymphocytes was determined by scoring a minimum of 500 lymphocytes in the cell mixture before the addition of agarose. After incubation under agarose and staining, 500 lymphocyte-target cell conjugates were observed, and the proportion of conjugates with non-viable (stained) target cells was determined. Target cell viability under these cultural conditions was checked in control dishes from which PBL were omitted.

RESULTS

Kinetics of ^{51}Cr release and IFN in the SCMC assay

Fresh PBL from a young adult pig and both PK-15-TGE and PK-15 target cells were used in the ^{51}Cr release assay. Supernatants were harvested at intervals of 0, 4, 8, 12 and 16 h and tested for ^{51}Cr release and IFN (antiviral) activity (Fig. 1). Neither significant specific ^{51}Cr release nor IFN activity were detected at 4 h, and little activity was demonstrated at 8 h. Both activities increased in parallel between 8 and 16 h. Higher levels of both specific ^{51}Cr release and IFN were obtained in the cultures containing PK-15-TGE target cells than in those containing uninfected PK-15 cells.

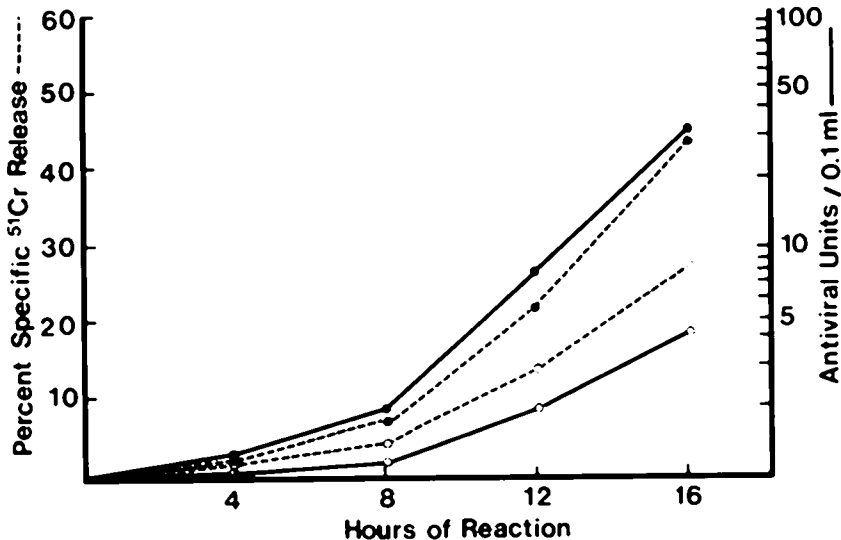


Fig. 1. Kinetics of specific ^{51}Cr release (-----) and interferon production (—) in the ^{51}Cr release assay for spontaneous cell-mediated cytotoxicity. The effector cells were peripheral blood lymphocytes from an adult pig, and the target cells were PK-15-TGE (●) or PK-15 cells (○).

Production of IFN by co-cultivation of target and effector cells

PBL from young adults and newborn piglets were compared with respect to IFN production in mixed cultures of effector cells and PK-15 and PK-15-TGE target cells. Three replicates of each test were performed, and the results are shown in Table I. It was confirmed that co-cultivation of PBL from the adult donors with PK-15-TGE cells resulted in the appearance of IFN activity in the media. Co-cultivation of adult PBL with the uninfected PK-15 cells also produced IFN, but the yields were significantly ($P < 0.05$) lower by the *t*-test than when PK-15-TGE cells were used. The mixed cultures of target cells with PBL from newborn piglets yielded comparable levels of IFN to the cultures containing adult PBL. IFN was not detected in the control cultures. One sample of medium from the co-cultivation of PK-15-TGE cells with adult PBL, containing 320 units ml⁻¹ of antiviral IFN activity, was used as a source of porcine IFN in the experiments described below.

TABLE I

Interferon titres in mixed cultures of lymphocytes and target cells

Target cells	Lymphocyte donor	Interferon titre ^a (units ml ⁻¹)
PK-15-TGE	Adult	533.4 ± 184.8
PK-15	Adult	46.7 ± 30.6
PK-15-TGE	Newborn	426.7 ± 184.8
PK-15	Newborn	33.4 ± 11.5
PK-15-TGE	None	0
PK-15	None	0
None	Adult	0
None	Newborn	0

^aMean ± standard deviation based on 3 replicates.

Effect of IFN on components of the SCMC reaction

PBL from a young adult pig were incubated with IFN (2×10^6 cells and 320 antiviral units ml⁻¹ IFN) or with a mock IFN control preparation obtained from lymphocytes cultured in the absence of target cells, for 18 h at 37°C and washed twice. The lymphocytes were then used in the SCMC ⁵¹Cr release assay against both PK-15 and PK-15-TGE target cells. In addition, mock IFN-treated PBL were tested against PK-15-TGE cells with 320 units ml⁻¹ IFN added directly to the reaction. As shown in Fig. 2, pretreatment of the effector PBL with IFN augmented their SCMC effector activity for both PK-15 and PK-15-TGE targets. However, the addition of IFN to the mixture of PK-15-TGE cells and PBL at the start of the reaction did not increase specific SCMC ⁵¹Cr release.

In the next experiment, PK-15 and PK-15-TGE target cells were pretreated with IFN or with a mock IFN preparation as above, and adult PBL pretreated with IFN were used as effector cells in the SCMC ^{51}Cr release assay. Pretreatment of the PK-15-TGE cells with IFN had no effect on the SCMC reaction (Fig. 3), while IFN pretreatment of the PK-15 targets reduced their release of ^{51}Cr in the SCMC reaction.

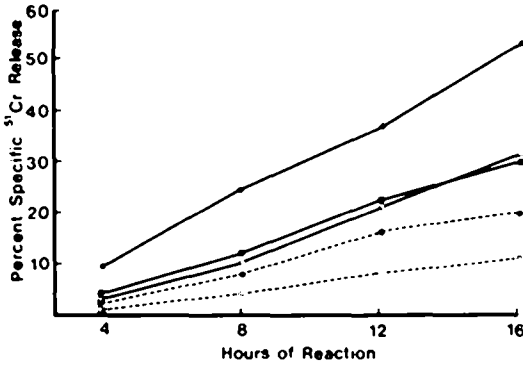


Fig. 2. Effect of interferon (IFN) pretreatment of adult peripheral blood lymphocyte (PBL) effector cells on specific ^{51}Cr release in the ^{51}Cr release assay for spontaneous cell-mediated cytotoxicity. IFN treated PBL + PK-15-TGE targets (●—●); mock IFN treated PBL + PK-15-TGE targets (○—○); mock IFN treated PBL + PK-15-TGE targets + IFN added to reaction (●—●); IFN treated PBL + PK-15 targets (●····●); mock IFN treated PBL + PK-15 targets (○····○).

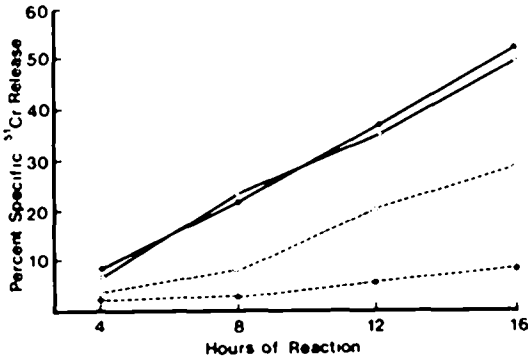


Fig. 3. Effect of interferon (IFN) pretreatment of target cells on specific ^{51}Cr release in the ^{51}Cr release assay for spontaneous cell-mediated cytotoxicity. The effector cells were IFN treated adult porcine peripheral blood lymphocytes. IFN treated PK-15-TGE targets (●—●); mock IFN treated PK-15-TGE targets (○—○); IFN treated PK-15 targets (●····●); mock IFN treated PK-15 targets (○····○).

The effect of IFN on SCMC was also studied by means of the single cell cytotoxicity assay. This was conducted with PBL isolated from neonatal piglets, as well as from adults, as effector cells, and with PK-15 and PK-15-TGE target cells. Both IFN pretreated (as above) and untreated target and effector cells were used. Four replicates of the experiment were done,

with PBL from different animals. The results are shown in Table II. PBL from newborn piglets bound to either target cell with the same frequency as PBL from SCMC competent adult pigs, and IFN treatment of either effector or target cells had no effect on target-binding frequency. The stimulatory effect of pretreatment with IFN on the SCMC effector activity of adult PBL for both PK-15-TGE and PK-15 cells was confirmed, since the mean percentages of lysed targets were significantly greater ($P < 0.05$) by the *t*-test when IFN pretreated PBL were used. Untreated PBL from newborn piglets failed to mediate SCMC against either target cell, but they were rendered cytotoxic by pretreatment with IFN. IFN pretreatment of PK-15-TGE did not influence their susceptibility to SCMC, while, as in the ^{51}Cr release assay, IFN pretreatment of the uninfected PK-15 cells resulted in significant ($P < 0.05$) protection against SCMC.

TABLE II

Effect of interferon (IFN) pretreatment of the effector peripheral blood lymphocytes (PBL) and target cells on binding and lytic capacity as determined by a single cell cytotoxicity assay

Source of PBL	Pretreatment of PBL	Targets	Pretreatment of targets	% PBL forming conjugates ^a	% conjugates with killed targets ^a
Adult	IFN	PK-15-TGE	None	9.7 ± 1.8	29.5 ± 3.2
		PK-15-TGE	IFN	10.0 ± 2.6	28.7 ± 4.3
		PK-15	None	9.6 ± 2.4	18.2 ± 5.6
		PK-15	IFN	10.3 ± 2.5	4.6 ± 1.2
	None	PK-15-TGE	None	9.4 ± 2.3	18.8 ± 4.1
		PK-15-TGE	IFN	9.7 ± 2.1	18.4 ± 3.3
		PK-15	None	9.5 ± 2.6	10.3 ± 1.9
		PK-15	IFN	9.6 ± 2.0	3.1 ± 0.9
Newborn	IFN	PK-15-TGE	None	9.4 ± 3.1	12.0 ± 1.6
		PK-15-TGE	IFN	10.1 ± 4.2	12.3 ± 1.8
		PK-15	None	9.3 ± 2.8	4.5 ± 1.3
	None	PK-15	IFN	9.6 ± 2.1	0
		PK-15-TGE	None	9.8 ± 1.9	0
		PK-15	None	10.4 ± 1.7	0

^aMean ± standard deviation based on 4 replicates.

DISCUSSION

The results show that the increase of specific ^{51}Cr release during the SCMC reaction was paralleled by the production of IFN, and that IFN was produced by the co-cultivation of monolayers of the target PK-15 or PK-15-TGE cells with the PBL effector cells. More IFN resulted from the mixed cultures containing TGEV-infected target cells than from those containing uninfected PK-15 cells. Similar results were obtained by Trinchieri et al. (1977, 1978) and by Santoli et al. (1978) with human lym-

phocytes and various virus-infected cells. The finding that PBL from newborn piglets, which we have shown previously to be unable to mediate SCMC (Cepica and Derbyshire, 1984a), bind to target cells with the same relative frequency as those from older SCMC-competent pigs, and respond by IFN production in mixed cultures, suggests that the lack of SCMC effector activity in newborn piglets is not associated with a lack of effector cells or their inability to produce IFN, but possibly with a lack of the stimulation needed for autologous IFN production *in vivo*.

Pretreatment of PBL with IFN was shown to augment their SCMC effector activity. This was observed for adult pigs in both the ^{51}Cr release assay and the single cell cytotoxicity assay. The promotional effect of IFN on SCMC effector activity is well established (Gidlund et al., 1978; Trinchieri et al., 1978; Djeu et al., 1979; Charley et al., 1983; Martin and Wardley, 1984), although in the porcine system, neither the type of effector cells nor the type of IFN involved have been determined, in contrast to human and murine systems (Rager-Zisman and Bloom, 1985). Of greater significance was our finding that lymphocytes from newborn piglets, normally inactive as SCMC effectors, could also be stimulated by IFN, though to a lesser degree than lymphocytes from SCMC competent older pigs. This observation suggests that it might be possible to stimulate SCMC incompetent lymphocytes in neonates *in vivo* by the treatment of piglets with IFN or IFN inducers.

The effects of IFN on the target cells in the SCMC reaction was also of interest. This was shown to be selective, in that the TGEV-infected PK-15 cells were not affected in their susceptibility to SCMC by pretreatment with IFN, whereas the uninfected PK-15 cells were protected against SCMC by IFN pretreatment. It is apparent that the effects of IFN were not mediated at the level of effector-target recognition, since the percentage of target-binding lymphocytes was similar, irrespective of IFN pretreatment of either target or effector cells. It could be speculated that if IFN acts in a similar manner *in vivo* on target and effector cells, it could be a powerful tool in the regulation of SCMC, by stimulating SCMC against virus-infected cells and protecting uninfected cells against SCMC.

ACKNOWLEDGEMENTS

This study forms part of a thesis submitted by the senior author to the Faculty of Graduate Studies, University of Guelph, in partial fulfillment of the requirements for the Ph.D. degree. The work was supported by the Ontario Ministry of Agriculture and Food and the Natural Sciences and Engineering Research Council of Canada. We also wish to acknowledge the technical assistance of Janis Milliken.

REFERENCES

- Cepica, A. and Derbyshire, J.B., 1983. Antibody-dependent cell-mediated cytotoxicity and spontaneous cell-mediated cytotoxicity against cells infected with porcine transmissible gastroenteritis virus. *Can. J. Comp. Med.*, 47: 298-303.
- Cepica, A. and Derbyshire, J.B., 1984a. Antibody-dependent and spontaneous cell-mediated cytotoxicity against transmissible gastroenteritis virus infected cells by lymphocytes from sows, fetuses and neonatal piglets. *Can. J. Comp. Med.*, 48: 258-261.
- Cepica, A. and Derbyshire, J.B., 1984b. The effect of adoptive transfer of mononuclear leukocytes from an adult donor on spontaneous cell-mediated cytotoxicity and resistance to transmissible gastroenteritis in neonatal piglets. *Can. J. Comp. Med.*, 48: 360-364.
- Charley, B., Petit, E., Laude, H. and La Bonnardière, C., 1983. Myxovirus- and coronavirus-induced in vitro stimulation of spontaneous cell-mediated cytotoxicity by porcine blood leukocytes. *Ann. Virol.*, 134E: 119-126.
- Djeu, J.Y., Heinbaugh, J.A., Holden, H.T. and Herberman, R.B., 1979. Augmentation of mouse natural killer cell activity by interferon and interferon inducers. *J. Immunol.*, 122: 175-181.
- Gidlund, M., Orn, A., Wigzell, H., Senik, A. and Gresser, I., 1978. Enhanced NK activity in mice injected with interferon and interferon inducers. *Nature*, 273: 759-761.
- Grimm, E. and Bonavida, B., 1979. Mechanisms of cell-mediated cytotoxicity studied at the single cell level. I. Estimation of cytotoxic T lymphocyte frequency and relative lytic efficiency. *J. Immunol.*, 123: 2861-2869.
- Kim, Y.B., Huh, N.D., Koren, M.S. and Amos, D.B., 1980. Natural killing (NK) and antibody-dependent cellular cytotoxicity (ADCC) in specific pathogen-free (SPF) miniature swine and germfree piglets. I. Comparison of the NK and ADCC. *J. Immunol.*, 125: 755-762.
- Martin, S.W. and Wardley, R.C., 1984. Natural cytotoxicity detected in swine using Aujeszky's disease virus infected targets. *Res. Vet. Sci.*, 37: 211-218.
- McClurkin, A.W. and Norman, J.O., 1966. Studies on transmissible gastroenteritis of swine. II. Selected characteristics of a cytopathogenic virus common to five isolates from transmissible gastroenteritis. *Can. J. Comp. Med.*, 30: 190-198.
- Norley, S.G. and Wardley, R.C., 1983. Investigation of porcine natural-killer cell activity with reference to African swine-fever virus infection. *Immunology*, 49: 593-597.
- Rager-Zisman, B. and Bloom, B.R., 1985. Interferons and natural killer cells. *Br. Med. Bull.*, 41: 22-27.
- Santoli, D., Trinchieri, G., Moretta, L., Zmijewski, C.M. and Koprowski, H., 1978. Spontaneous cell-mediated cytotoxicity in humans. Distribution and characterization of the effector cell. *Clin. Exp. Immunol.*, 33: 309-318.
- Trinchieri, G., Santoli, D., Zmijewski, C.M. and Koprowski, H., 1977. Functional correlation between antibody-dependent and spontaneous cytotoxic activity of human lymphocytes and possibility of an HLA-related control. *Transpl. Proc.*, 9: 881-884.
- Trinchieri, G., Santoli, D., Dee, R.R. and Knowles, B.B., 1978. Antiviral activity induced by culturing lymphocytes with tumor derived at virus transformed cells. Identification of the antiviral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J. Exp. Med.*, 147: 1299-1330.