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B. Charley, Élisabeth Petit, C. La Bonnardière. INTERFERON-INDUCED ENHANCEMENT OF NEWBORN PIG NATURAL KILLING (NK) ACTIVITY. Annales de Recherches Vétérinaires, 1985, 16 (4), pp.399-402. hal-00901601

HAL Id: hal-00901601
https://hal.science/hal-00901601

Submitted on 11 May 2020

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Ann. Rech. Vét., 1985, **16** (*4*), 399-402

INTERFERON-INDUCED ENHANCEMENT OF NEWBORN PIG NATURAL KILLING (NK) ACTIVITY

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Résumé

AUGMENTATION, INDUITE PAR L'INTERFÉRON, DE L'ACTIVITÉ NK DES PORCELETS NOUVEAU-NÉS. — La possibilité d'activer la fonction NK («Natural killing», ou cytotoxicité spontanée) par l'interféron, a été étudiée à partir de leucocytes sanguins de porcelets nouveau-nés. Alors que cette fonction est très peu développée chez des porcelets âgés de moins d'une semaine, elle se trouve significativement accrue si les leucocytes de ces animaux sont incubés *in vitro* en présence d'interféron α humain semi-purifié. Ceci suggère que les porcelets ont des cellules «pré-NK» qui acquièrent leur pleine capacité cytotoxique après traitement par l'interféron.

Natural killing (NK) activity refers to the ability of normal leukocytes to kill tumor as well as virus-infected cells *in vitro*. NK activity receives much attention especially because it could represent a non-specific defence mechanism against tumors and infectious diseases (Herberman and Ortaldo, 1981). In porcine species, NK activity was described by using various human tumor cell lines (Koren *et al.*, 1978; Leibold *et al.*, 1980; Charley *et al.*, 1983; Norley and Wardley, 1983) and virus infected porcine cells (Cepica and Derbyshire, 1983) as target cells. Concerning porcine infectious diseases, several viruses were shown to modulate porcine NK; thus, virulent swine Influenza virus, virulent and inactivated Transmissible Gastroenteritis Virus (TGEV) increased NK *in vitro* (Charley *et al.*, 1983; Laude *et al.*, 1984) whereas african swine fever virus-infected pigs had decreased NK (Norley and Wardley, 1983). Influenza virus and TGEV-induced NK enhancement was found related to endogenously produced leukocyte interferon (IFN) (Charley *et al.*, 1983). Moreover, crude porcine IFN or semi-purified human α IFN were shown *in vitro* to boost adult pig NK activity (Charley *et al.*, 1983). Since NK was found in very or undetectable in newborn pigs (Huh *et al.*, 1981), we decided to look at the *in vitro* effects of semi-purified IFN on newborn porcine NK activity. In the present report, we show that preincubation of leukocytes with α IFN can stimulate low NK activity observed in newborn piglets.

Materials and Methods

Animals

Two litters of naturally-farrowed, colostrum-fed Large-White piglets, raised in INRA-La Minière facilities, were used for these experiments.

Preparation of porcine leukocytes

Mononuclear cells from peripheral blood were prepared by sedimentation of red blood cells in Dextran (1 vol. 50 mg/ml Dextran 200 from Serva, Heidelberg, RFA, + 3 vol. of blood) followed by centrifugation over a Ficoll-Telebrix mixture (Salmon, 1979).

Assay for NK

A 4 hours ^{51}Cr-release assay was used as previously described (Koren *et al.*, 1978; Charley *et al.*, 1983) with human leukemia K562 cells as target cells and overnight preincubated porcine leukocytes as effector cells. Results were expressed as % cytotoxicity (Koren *et al.*, 1978).

Interferon

Human α IFN was a generous gift from Institut Pasteur Production (Garches, France). It was produced in Sendaï virus infected leukocytes and semi-purified according to Cantell *et al.* (1978). Its specific activity was 10^6 IU/mg of protein. IFN was incubated overnight with leukocytes (plated at a density of 5×10^6 cells/ml, corresponding to an effector to target ratio of 50:1) before NK assay, as described previously (Charley *et al.*, 1983).

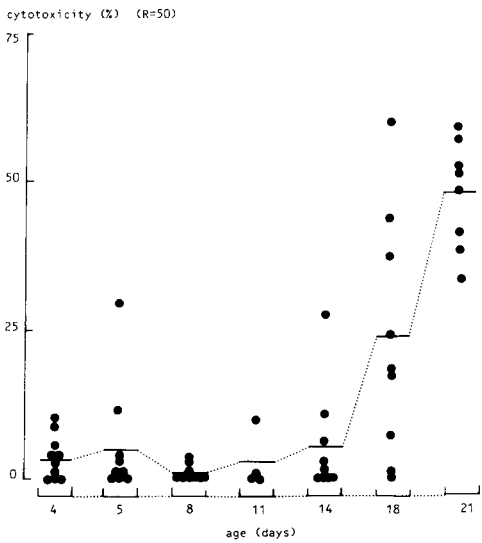

Fig. 1. — Post-natal development of NK activity: leukocytes from 10 piglets of a same litter were tested in a 4 hours ^{51}Cr-release assay against K562 cells. Effector to target ratio of 50:1.

Results and Discussion

In the first experiment, NK activity was assayed in 10 piglets from one litter: our results (fig. 1) confirmed previous data from Huh *et al.* (1981), showing that NK activity was very low during the first week of life and gradually increased during following weeks. Interestingly, Huh *et al.* (1981) observed that NK post-natal development was under environmental influences, since NK remained very low in 3 week-old germ-free piglets. As our previous observations indicated that 2×10^4 IU (International Units) of human α IFN per ml stimulated adult porcine NK (Charley *et al.*, 1983), we pretreated 4 day-old pig leukocytes with a same amount of human α IFN and observed a significantly increased cytotoxicity (table 1). This experiment was extended to a second litter for which NK was assayed from 6 to 43 days of age: in this case, NK was also stimulated when leukocytes were preincubated with 2×10^4 IU/ml of IFN (fig. 2).

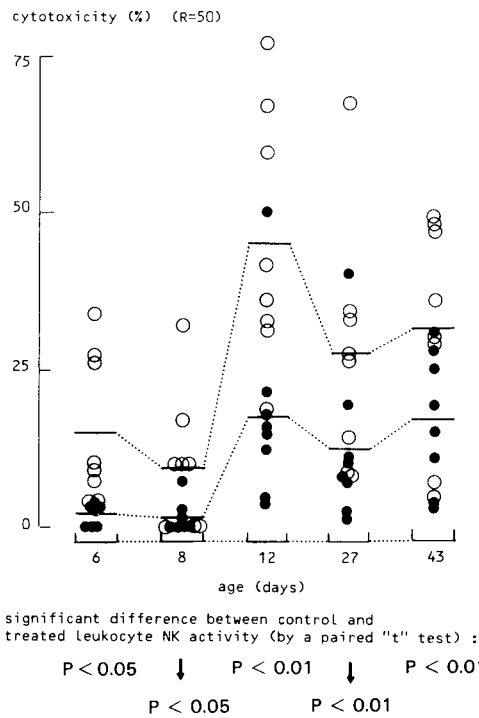

Fig. 2. — Post-natal development of NK activity: effect of incubation of leukocytes with human α IFN. Black symbols: control leukocytes. Open symbols: IFN pretreated leukocytes (effector to target ratio of 50:1).

Table 1. — *In vitro* effect of human αIFN on 4 day old pig leukocyte NK activity, tested at an effector to target ratio of 50 : 1

Pig no.	NK (% cytotoxicity)	
	Control leukocytes	IFN-treated leukocytes[1]
55	0	0
51	0	0
57	0	0.4 ± 0.3
53	1.5 ± 0.1	4.5 ± 0.3
50	2.8 ± 0.2	2.1 ± 0.2
52	3.9 ± 0.6	8.1 ± 0.3
56	3.9 ± 0.9	9.4 ± 2.1
64	5.8 ± 0.2	25.2 ± 0.3
54	9.0 ± 0.8	17.6 ± 1.4
61	10.4 ± 1.2	29.3 ± 1.1

1. The difference between control and treated NK was significant ($P < 0.05$, by a paired «t» test).

Therefore these results indicated that, although newborn pig leucocytes exhibited a very low NK activity, they were susceptible to IFN boosting effect, starting from at least day 4 after birth. For several years, IFN has been shown to enhance NK, mainly by recruiting non-cytolytic pre-NK cells into mature cytotoxic NK cells and by accelerating kinetics of lysis (reviewed by Saksela, 1981).

In a study on human newborn NK, although 30% of cord blood leukocyte preparations were devoid of NK, all suspensions tested had a clearly increased NK activity upon exposure to IFN (Antonelli *et al.*, 1981). Our results are in agreement with this last report and therefore suggest that newborn piglets have pre-NK cells which become cytotoxic when treated by IFN. These data could also support the proposition of Huh *et al.* (1981) that environmental microbial stimuli would induce IFN which in turn could activate newborn pig NK.

Further studies on the possible NK activation by IFN treatment of newborn piglets may provide greater understanding of the biological role of NK and IFN in resistance to neonatal viral diseases.

Received, 30th January, 1985.
Accepted, 15th March, 1985.

Acknowledgements

We thank M. Bonneau, from INRA La Minière, for providing Large White-piglets.

Summary

The possible activation of Natural Killing (NK) activity by interferon (IFN) was studied in peripheral blood leukocytes of newborn piglets. It is reported that, although NK is very low during the first week of life, *in vitro* pretreatment of newborn leukocytes with semi-purified human α IFN induces a significant NK enhancement. These data suggest that piglets have pre-NK cells which become fully cytotoxic when exposed to IFN.

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