Absence of porcine interferon alpha secreting cells in severe combined immunodeficiency (SCID) mice inoculated with porcine leukocytes

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Short note

Absence of porcine interferon alpha secreting cells in severe combined immunodeficiency (SCID) mice inoculated with porcine leukocytes

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Abstract – A low frequency leukocyte subpopulation, referred to as natural interferon producing cells (NIPC) is able to produce high amounts of interferon α (IFN-α) following contact with non-infectious viral structures. In order to examine the possible leukocytic nature and bone marrow origin of NIPC, severe combined immunodeficiency (SCID) mice were reconstituted with porcine leukocyte populations, including bone marrow cells. At different times after reconstitution, enriched CD4 and CD45 positive porcine cells were isolated from various mouse organs and tested for the presence of porcine NIPC by porcine IFN-α specific ELISPOT assay, after in vitro stimulation by UV inactivated transmissible gastroenteritis virus (TGEV). Although engraftment of porcine cells in SCID mice was shown by flow cytometry and by the production of pig immunoglobulins, no IFN-α secreting cells could be detected. This result suggests that NIPC do not derive from bone marrow precursor cells, or that growth factors needed for in vivo expansion of porcine NIPC were absent in mice. © Inra/Elsevier, Paris.

interferon / TGEV / pig / mice

Résumé – Absence de cellules sécrétrices d'interféron alpha porcin chez des souris immunodéficientes SCID recevant des leucocytes de porc. Une sous-population leucocytaire très minoritaire, appelée cellules productrices naturelles d'interféron (NIPC en anglais) produit de fortes quantités d'interféron alpha (IFN-α) à la suite d'un contact avec des structures virales non infectieuses. Afin de déterminer si cette population cellulaire est de nature leucocytaire et a une origine médullaire, des souris immunodéficientes (SCID) ont été reconstituées par plusieurs populations leucocytaires porcines, dont des cellules de moelle osseuse. À différents temps après injection de ces cellules, la présence de cellules NIPC a été recherchée dans plusieurs organes lymphoïdes de souris, au moyen d'un test immunoenzymatique Elispot. Celui-ci a été réalisé sur des cellules enrichies en cellules

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porcines $CD4^+$ et $CD45^+$ par tri magnétique, après incubation avec le virus de la gastroentérite transmissible (VGET), inactivé par les rayons U.V. Bien que le succès de la greffe de cellules porcines chez les souris SCID ait été attesté par une analyse en cytométrie de flux et par la production d'immunoglobulines de porc, nous n'avons pu détecter de cellules sécrétrices d'IFN-α. Ce résultat suggère que les cellules NIPC ne dérivent pas de précurseurs médullaires, ou que des facteurs de croissance nécessaires au développement in vivo des cellules NIPC de porc sont absents chez la souris.

interferon / VGET / porc / souris

1. INTRODUCTION

Type I interferons (IFN-α/β) constitute an early and essential host response to viruses. A low frequency subpopulation of leukocytes, referred to as natural interferon producing cells (NIPC), is able to produce high amounts of IFN-α following short exposure to non-infectious viral structures. NIPC were characterized as non-adherent, non-T, non-B, CD 4^+ and MHC class II^+ cells (reviewed in [8]). A recent thorough flow cytometry analysis of human blood IFN-α secreting cells (IFN-α SC) indicated that they share several cell surface markers with immature dendritic cells [21]. Although NIPC are primarily found in lymphoid tissues [1, 7, 14, 15, 20] their origin and precise lineage remain to be determined. Their hematopoietic origin was suggested by in vivo studies in which bone-marrow engraftment in irradiated pigs was followed by the reconstitution of a circulating IFN-α SC population [6].

Severe combined immunodeficiency (SCID) mice, first described by Bosma et al. [4], are characterized by a defective recombination of antigen receptor genes in both B and T lympocytes. As a consequence, they show a poor ability to reject allogeneic or xenogeneic graft and have therefore been used as small animal models in which xenogeneic immune systems were reconstituted [2, 3, 9, 13, 18].

In order to determine the hematopoietic origin of NIPC, the present work was undertaken to determine if porcine IFN-α SC were present in SCID mice reconstituted with a porcine immune system.

2. MATERIALS AND METHODS

2.1. Animals

Forty-seven 10–12-week-old SCID mice of both sexes, selected on the basis of serum immunoglobulin (Igs) concentrations lower than 20 μg/mL, were used in this experiment. Titrations of murine Igs were performed by sandwich ELISA using polyclonal pig anti-mouse antibodies (Sevac, Prague, Czech Republic). Mice were kept in our breeding facilities, in a fibreglass isolator with filtered air. They were fed gamma-irradiated commercial pellets and autoclaved water with vitamin addition.

Twenty mice were used after 3Gy gamma irradiation and 27 mice were non-irradiated. Porcine cells were injected intraperitoneally (i.p.) into mice 3 days after the gamma irradiation.

2.2. Preparation of porcine cells

Peripheral blood leukocytes (PBL), splenocytes and bone-marrow cells were obtained from five 3-month-old outbred miniature pigs bred in our laboratory. Blood was diluted 3:1 by 6 % dextran in phosphate buffer saline (PBS), and kept for 1 h at room temperature in a 45 ° angle position. Buffy coat was removed, diluted in PBS and centrifuged. Cells were washed in PBS and erythrocytes lysed by the addition of deionized water. Intraperitoneal injection was performed using 3×10^6 to 10×10^6 PBL into irradiated and non-irradiated mice. Spleen was minced in cold PBS, cell debris was removed by 5 min sedimentation at 1 *g* and cells washed three times in PBS. Both femurs and sternum were cut by scissors and bone marrow cells prepared as for spleen.

Then, 4×10^7 to 6×10^7 splenocytes and 6×10^7 bone-marrow cells were injected i.p. in groups of four mice.

2.3. Porcine Ig quantification

Porcine Ig concentrations were estimated in mouse sera by sandwich ELISA. Capture polyclonal rabbit anti-swine antibodies (Sevac, Prague, Czech Republic) were bound to ELISA 96-well microplate (Gama, Prague, Czech Republic). PBS with 0.5 % BSA was used as saturation step and as diluting solution. Peroxidase conjugated rabbit anti-swine antiserum (Sevac, Prague, Czech Republic) and tetramethylbenzidine (TMB) substrate were used. Calibrated pig serum with predetermined total Ig concentration, used as a standard, was kindly donated by Dr Helena Tlaskalova (Institute of Microbiology, Prague, Czech Republic). The linear part of the calibration curve with the lowest limit of sensitivity of 20 ng/mL of porcine total Igs was used. The absence of cross reactivity with murine Igs was tested using diluted mouse blood plasma with Ig concentration of 1 mg/mL.

2.4. Preparation of mouse cells

Mice were bled by cardial puncture under anaesthesia. Cell suspensions from liver, femur and sternum marrow, and spleen were prepared by cutting the organs in cold PBS. Cells were collected after 5 min sedimentation. Peritoneal cells were obtained by washing the peritoneal cavity with PBS. PBL were obtained by lysis of blood with deionized water. Cells were washed three times in PBS.

2.5. Separation of porcine $CD4^+$ or $CD45^+$ cells from porcine and murine cells mixture

Cells (5×10^5) were incubated with anti-porcine CD4 monoclonal antibody (mAb) 10-2H2 and anti-CD45 mAb K252.1E4 [16] for 1 h in cold PBS with 1 % BSA. Cell suspensions were washed three times in cold PBS–BSA. Incubation with streptavidin conjugated Dynabeads M-280 (Dynal, Oslo, Norway) was performed in PBS with 0.5 % BSA for 1 h at 4 °C. CD4 and CD45 positive porcine cells were magnetically separated and washed three times in RPMI 1640 with 15 % fetal calf serum (FCS) before use in porcine IFN-α-specific ELISPOT assay.

2.6. Virus

The Purdue 115 strain of transmissible gastroenteritis virus (TGEV) was produced and UV-inactivated as described before [11].

2.7. Porcine IFN-α-specific ELISPOT assay

Cells were incubated in 200 µL of RPMI 1640 with 10 % FCS, L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (0.1 mg/mL) in 5 % CO_2 atmosphere at 37 °C, in nitrocellulose-bottomed microplates MAHA N 45 (Millipore, Bedford, USA) coated with anti-pig IFN-α mAb K9 as described previously [12]. Production of porcine IFN-α was induced by addition of UV-inactivated TGEV and 16 h later IFN-α SC frequency was estimated by ELISPOT assay using peroxidase labelled anti-pig IFN-α mAb F17 [12]. Spots were counted under binocular microscope.

2.8. Cell staining and flow cytometry analysis

Fluorescent mouse mAbs against pig leukocyte markers K252.1E4 (anti-CD45, IgG1) and 74-22-15A (anti-granulocyte/macrophage SWC3, Ig2b) [16] were used. Cells were washed in PBS–1 % BSA (with 0.1 % NaN_3), incubated in PBS–NMS (10 % heat-inactivated normal mouse serum and 0.1 % NaN_3 in PBS), washed and incubated for 30 min with FITC-labelled mAb. Samples were analysed with a FACSort (Becton-Dickinson) or EPICS ELITE (Coulter) flow cytometer. At least 10 000 events were collected and only propidium iodide negative cells were gated and analysed.

3. RESULTS

When SCID murine and porcine cells were mixed before IFN-α induction, we observed that murine cells exerted an inhibitory effect on IFN-α secretion by porcine cells (*table I*). In order to allow the detection of IFN-α SC by the ELISPOT assay, CD4 and CD45 positive porcine cells were therefore magnetically isolated from

mouse organs, before IFN-α induction, in all subsequent experiments (data not shown).

SCID mice were divided into two groups (gamma-irradiated and non-irradiated mice). At different times (from 3 to 9 weeks) after i.p. injection of porcine PBL, bone-marrow, liver or spleen cells were collected and ELISPOT assay was performed on $CD4^+$ $CD45^+$-enriched porcine cells. The low number of magnetically separated cells from mouse organs did not allow flow cytometry analysis of their content. All cells prepared from one organ were tested in one well of ELISPOT assay. At no time after i.p. injection, in either irradiated or non-irradiated SCID mice, could any positive porcine IFN-α SC be found after in vitro TGEV induction, whatever the type of porcine cells injected into SCID mice, or the mouse organ tested.

In order to verify if injected SCID mice were indeed reconstituted with porcine lymphoid cells, the presence of porcine cells and porcine Igs in SCID mice was tested by flow cytometry and porcine Ig-specific ELISA. Porcine Igs were detected in injected SCID mice serum (*table II*), with concentrations up to 20 μg/mL. By flow cytometry using porcine pan leukocyte (CD45) and granulocyte/macrophage (SWC3)-specific mAbs, a low percentage of porcine cells (0–5.1 %) was found in mouse organs (*table III*).

Table I. Influence of SCID mouse cells on porcine IFN-α secreting cell (SC) frequency.

Murine cells (10^4/mL)	Porcine/murine cells ratio	IFN-α SC[a]
1.5	128	65
3	64	61.5
6	32	62
12	16	48
25	8	25
50	4	18
100	2	8.5
200	1	3.3

Porcine PBL at a constant concentration (2×10^6/mL) were mixed with increasing amounts of mouse SCID cells, before TGEV induction.
[a] IFN-α SC frequency: number of IFN-α SC per 10^5 cells as measured by ELISPOT assay.

4. DISCUSSION

The aim of the present work was to determine if porcine IFN-α SC were present in SCID mice following their reconstitution with porcine immune cells. Although a successful engraftment of porcine cells in SCID mice was demonstrated by porcine CD45 and SWC3 flow cytometry analysis, and by porcine Ig quantification in mouse serum, no IFN-α SC could be shown in reconstituted SCID mice.

Table II. Porcine Ig concentration (ELISA) in the serum of porcine cells reconstituted SCID mice.

Weeks after injection of porcine cells	Non-irradiated mice		Irradiated mice	
	n[a]	Ig (μg/mL)	n	Ig (μg/mL)
3	5/5	11.2 ± 5.7[b]	5/5	12.6 ± 7.7
4	4/4	14.3 ± 7.0	4/4	15.3 ± 8.5
5	4/5	12.4 ± 6.5	4/5	19.6 ± 14.8
7	5/7	9.8 ± 4.7	3/3	11.9 ± 5.5
9	5/6	6.9 ± 3.5	3/3	5.8 ± 2.7

[a] n: number of porcine Ig-positive mice/total number of SCID mice studied.
[b] Results are expressed as mean ± standard deviation.

Table III. Percentage of porcine cells in different organs of reconstituted SCID mice.

Weeks after injection of porcine cells	Mouse organ	Non-irradiated mice			Irradiated mice		
		n	CD 45$^+$	SWC3$^+$	n	CD 45$^+$	SWC3$^+$
3	bone marrow	5	0.55–0.90	ND	5	0.20–3.05	ND
	spleen	5	0.05–0.10	ND	5	0.00–0.05	ND
	blood	5	0.05–0.10	ND	4	0.15–0.50	ND
	peritoneum	4	0.10–0.60	ND	5	0.20–3.00	ND
4	bone marrow	4	0.30–5.10	0.30–0.60	4	0.25–0.35	0.10–0.30
	spleen	4	0.05–0.40	0.05–0.40	4	0.00–0.10	0.00–0.10
	blood	4	0.20–0.60	0.20–0.60	4	0.05	0.05
	peritoneum	4	0.10	0.10	4	0.10–4.00	0.10–2.50
5	bone marrow	5	0.20–0.30	0.10–0.20	5	0.20–2.50	0.10–0.25
	spleen	4	0.05–0.20	0.05–0.20	5	0.05–0.20	0.05–0.20
	blood	5	0.30–0.70	0.20–0.50	5	0.35–0.70	0.30–0.50
	peritoneum	5	0.10–0.30	0.10–0.20	5	0.10–0.80	0.10–0.60
7	bone marrow	7	0.00–0.10	0.05	3	0.10–0.30	0.05
	spleen	6	0.15	ND	3	0.05–0.20	0.05–0.10
	blood	6	0.00	0.00	3	0.05	0.05
	peritoneum	0	ND	ND	0	ND	ND

CD45 is a marker for porcine leukocytes, and SWC3 a marker for granulocytes and macrophages.
Results expressed as range of percentages.

The number of porcine cells present in reconstituted SCID mice was low but comparable to what was described by others using pig cells [10, 13]. In addition, no positive effect of gamma irradiation on engraftment efficiency was observed, as shown by others [19].

There are several possible explanations for our inability to detect IFN-α SC in reconstituted SCID mice. 1) Engraftment efficiency may be too low to allow the detection of highly infrequent cells such as IFN-α SC. In our previous studies on porcine IFN-α SC, the ELISPOT assay used was shown to detect a minimum of one IFN-α SC per 10^5 cells [6, 12, 20]. The number of sorted CD4$^+$ CD45$^+$ porcine cells from mouse organs in the present study was low (around 10^5 cells) but probably sufficient to allow the detection of positive IFN-α SC. 2) NIPC may not derive from hematopoietic precursors, and would therefore be absent in mice reconstituted with a porcine immune system. This explanation is, however, unlikely since bone-marrow engraftment experiments in irradiated pigs have suggested a bone-marrow origin of IFN-α SC [6]. In addition, NIPC in several species were found to express several leukocyte markers [8, 12, 15, 21]. 3) Another possible explanation is that in vivo expansion of NIPC from porcine bone-marrow precursors could be inhibited by mouse cells, or would require homologous growth factors which were not present in SCID mice. Accordingly, hematopoietic factors such as GM-CSF or IL-3 were shown to exert a differentiation activity on human NIPC [5], and there may be little or no biological cross-reactivity of mouse hematopoietic factors on porcine cells.

Our observation of an inhibitory effect of mouse cells on IFN-α production by porcine cells may deserve further analysis. In the context of xenografts [17], it would indeed be important to determine to what extent and by which mechanisms xenogeneic cells may inhibit the production of important cytokines such as IFN-α by recipient cells.

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