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Short communication

IL-23/IL-17/G-CSF pathway is associated with granulocyte recruitment to the lung during African swine fever

Z. Karalyan*, H. Voskanyan, Z. Ter-Pogossyan, D. Saroyan, E. Karalova

Laboratory of Cell Biology and Virology, Institute of Molecular Biology of NAS RA, 0014 Yerevan, Armenia

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ABSTRACT

The interleukin (IL)-23/IL-17 pathway plays a crucial role in various forms of inflammation but its function in acute African swine fever (ASF) is not well understood. Thus, in this study, we aimed to find out whether IL-23/IL-17/G-CSF is released in acute ASF and what function it may have.

The present study revealed that the production of IL-17 and IL-23 were significantly increased in the sera of ASFV infected pigs. Using ELISA, we found that the serum levels of IL-23 and IL-17 have overexpressed in ASF virus infected pigs compared with healthy controls. The levels of IL-17 and IL-23 increase in the early stages and the levels of G-CSF and C – reactive protein in the later stages of ASF. Simultaneously, with the increase of the levels of IL-23/IL-17 extravasation of granular leukocytes in the tissue (diapedesis) is observed. Diapedesis can explain the neutropenia that we identified previously in the terminal stages of ASF.

The increase in serum levels of IL-23/IL-17 is preceded by enhanced migration of neutrophils in tissues, and the last one is preceded by neutropenia. The increase in serum levels of G-CSF has compensatory nature, directed on stimulation of proliferation of granulocytes. Taken together, our results revealed an overexpression of the IL-23/IL-17 axis in the ASF virus infected pigs, suggesting that it may be a crucial pathway in the diapedesis at ASF.

1. Introduction

The pathogen of African swine fever (ASF) is a DNA virus which is the sole member of the Asfarviridae family. In pigs, ASF virus (ASFV) predominantly replicates in the monocyte/macrophage lineage cells, although some other cell types also can be infected at the late stage of the disease. The infection of macrophages with several different ASFV strains leads to an increased secretion of proinflammatory cytokines (Gomez-Villamandos et al., 2013; Zakaryan et al., 2015).

Neutrophils are an essential component of innate immunity and host defense against various infections by virtue of their rapid response to different types of pathogens. Neutrophils also play an important role in tissue damage during inflammation (Mei et al., 2012). Under most inflammation types, neutrophils are the first cell type that can cross the blood vessel endothelium and migrate into the tissue (Lim et al., 2015). In our previous work, we reported severe neutropenia at the acute ASFV infection. Infections can cause neutropenia by damaging neutrophil production or by immune destruction or rapid recruitment of neutrophils by the tissue infiltration (Karalyan et al., 2012a). Proinflammatory signals emerge at the initial stage of sepsis, thus making possible circulating neutrophils reach sites of inflammation (Zakaryan et al., 2015). Since neutrophil infiltration is a significant component of several septic conditions, which is an early event in the progress of sepsis and septic shock (Cauvi et al., 2014), we investigated the possible mechanism causing neutrophil migration into the tissues.

Iwakura and Ishigame (2006) postulates that inflammatory responses could be triggered by 3 independent pathways: interleukin-12 (IL-12)/IFN-γ, IL-4/IL-5/IL-13, and IL-23/IL-17. In some infections, IL-17 produced by CD4+ T cells (T-helpers) and NKT cells has been revealed to increase host defense by the stimulation of proinflammatory chemokines, cytokines, and antimicrobials engaged in polymorphonuclear leukocyte recruitment (Peck and Mellins, 2010). Reports both in the mouse and the human model have revealed that IL-23 is significant for the expression and maintenance of T-helper (Th)17 (and production of IL-17) responses (Gagliardi et al., 2011). They associated with chronic inflammatory processes, autoimmune diseases, allergic response and transplant rejection. In addition, IL-23 may directly stimulate a subclass of macrophages and dendritic cells expressing IL-23R, causing the synthesis of inflammatory cytokines, such as TNF-α and IL-1

* Corresponding author.
E-mail address: zkaralyan@yahoo.com (Z. Karalyan).

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(Iwakura and Ishigame, 2006). Authors propose that IL-23 can stimulate autoimmune response through the induction of Th17/ThIL-17 cells; and/or activation of IL-1 and IL-6 synthesis via myelocytes activation. IL-23 plays a major role in autoimmune destruction in experimental allergic encephalomyelitis, collagen-induced arthritis inflammatory bowel disease, and some other autoimmune processes (McKenzie et al., 2006). It is good known fact that the elevated levels of cytokines such as TNF-a and IL-1β play a significant role in the pathogenesis of ASF because of their proinflammatory, and proapoptotic profile (Gomez-Villamandos et al., 2013; Zakaryan et al., 2015). One of the mechanisms involved in the failure to sustain self-tolerance in general autoimmune pathogenesis is polyclonal lymphocyte activation induced by several viral infections (Barcellini, 2015).

Characteristics of the IL-23/IL-17 dependent pathway and the activation of myeloid cells in ASF have not been sufficiently studied up to now. Immune hyperactivity traits are not always associated with autoimmune disorders, but autoimmune activity described in several viral hemorrhagic fevers (Ramírez-Fonseca et al., 2015).

Despite the importance of IL-23/IL-17 in the general pathology of the immune system, there is no study describing the IL23/IL17 status of pigs infected with ASFV genotype II. So the goal of this study was to investigate the consequence of ASFV genotype II on the host.

2. Materials and methods

2.1. Viral stock and animal experiment

The virus (Arm07) used in this study was isolated in 2007 from the spleen of a swine infected with ASFV. It belongs to genotype II, which is distributed in the Trans-Caucasian countries (Rowlands et al., 2008). In this study, fifteen pigs (n = 15) of the same age (3 months old) and weight (30–32 kg) were used for infection (n = 12) and as controls (n = 3). The animals were housed in separate stables, where they had access to a commercial feed twice per day and to clear water at all times. Pigs were infected by intramuscular injection with 104 50% hemadsorbing doses (HAD50)/ml. Nine (n = 9) pigs were used for tissue sampling and cell analysis, whereas three other pigs (n = 3) were used for blood collection and cytokine experiments. The viral titer in sera was determined by hemadsorption microtest as described previously and expressed as log10 HAD50/ml (Enjuanes et al., 1976; Carrascosa et al., 2011). The animal experiments were permitted by the Institutional Review Board/Independent Ethics Committee of the Institute of Molecular Biology of NAS RA (reference number IRB00004079).

2.2. Blood collection and ELISA

From 1 to 7 dpi, blood samples were collected from the ophthalmic venous sinus as described previously (Stier and Leucht, 1980). Preinoculation (0 dpi) blood samples were taken to obtain control values. In order to detect serum levels of CRP, (C-Reactive Protein), IL-17, IL-23 and GCSF commercial ELISA kits (Elabscience Biotechnology Co., Ltd) were used. The levels of cytokines (pg/ml) were measured using a colorimetric reader (Stat Fax 303 Plus) and calculated according to the cytokine standard curve supplied in the kits. All samples were tested in duplicate according to manufacturers' instructions.

2.3. Tissue samples

Samples from lungs were fixed in 10% buffered formalin solution (pH 7.2) for 24 h. After fixation, the samples were dehydrated through a graded series of alcohols, washed with xylol and embedded in paraffin wax by a routine technique for light microscopy. For structural analysis, wax-embedded samples were cut (Microm HM 355, 5 lm) and stained with hematoxylin and eosin in accordance with the manufacturer's protocol (Sigma-Aldrich, Germany). The histological examination was implemented using a light microscope.

2.4. Neutrophil quantification

The quantitation of neutrophil accumulation in lungs was conducted by direct evaluation as described previously (Bureau et al., 1980; Matute-Bello et al., 2011). Briefly, for each tissue sample, 12 serial sections were evaluated quantitatively with a micrometer eyepiece grid. Each square represented an area of 3780 $(70 \times 54)\,\mu m^2$ on the original section. Average cell counts were made successively for each area, each tissue block, each animal and each batch. Granulocytes and macrophages of the alveolar airspaces, alveolar walls, and interstitium are counted. Alveolar wall neutrophils contain cells entered the interstitium and those that are circulating or adhere to the alveolar capillaries (Matute-Bello et al., 2011).

2.5. Statistics

The received results were expressed as mean ± SD, or median if data were not normally distributed. All statistical analysis was performed using SPSS statistical software for Windows, version 17.0 (SPSS, Inc., Chicago, IL, USA). For continuous variables, statistical significance was calculated using Mann-Whitney *U* test. Non-parametric Spearman's rho test was used to assess the correlation of different variables. All hypotheses were 2-tailed, and p values <0.05 were considered significant.

3. Results

3.1. Experimental infection

ASF experimental infection described previously (Zakaryan et al., 2015) was characterized by early viraemia starting from 1 to 2 dpi. Viraemia peaked at 5 dpi (virus titers in blood were 5.0–5.25 log10 HAD50/mL). The high titres of ASFV were determined in all pigs up to 7 dpi.

3.2. Levels of serum cytokines

The concentrations of IL-17, IL-23, GCSF and CRP in serum collected from ASFV-infected pigs from 0 to 7 dpi are displayed in Fig. 1(A–D).

Pigs infected with ASFV presented a significant increase ($p < 0.05$) of IL-17 levels in the 1 st day of infection till the 3 dpi. Subsequently, IL-17 serum levels have returned to control values.

Upon infection, the level of serum IL-23 started to rise and reached a peak on the last day of the experiment. Significant changes were found between controls and infected animals from 1 dpi.

A significant elevation of GCSF ($p < 0.05$) was observed at 3 dpi and became about 4 times higher than in control on day 3. Afterward, GCSF serum levels have returned to control values.

The concentrations of CRP were generally higher in pigs with the clinical course of ASF. In ASFV-infected pigs, the mean concentration significantly increased from 3 to 4 dpi and remained elevated to 7 dpi ($p < 0.05$–$p < 0.01$). The concentration of IL-17 and IL-23 are preceded by high titers of ASFV observed from 3 dpi, and approximately coincides with the CRP levels (Fig. 1E).

Granular leukocytes migration or diapedesis (cellular extravasation) is critical for immune surveillance and a crucial first step in