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LETTER TO THE EDITOR

Virus dose-dependent neutrophil and lymphocyte proportions in peripheral blood during influenza A infection of mice

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The course of influenza infection varies depending on the interaction of the virus and the immune system. The mechanisms of both the innate and the specific immune response are involved in these interactions. Important role in the innate immune defense play neutrophils, which are involved in the recovery from the IAV infection (*1, 2, 3*). It was shown that neutrophils can also serve as antigen-presenting cells to antiviral $CD8^+$ T-cells (*4*). In this study, we focused on monitoring the changes in the proportion of cell types in the peripheral blood of mice infected with mouse-adapted human influenza A viruses (IAVs) (*5*). Our aim was to determine how the changes in the differential leukocyte count correlate with the virulence of IAV used for the infection. BALB/c mice were infected with sublethal dose of moderately virulent IAV strain A/Mississippi/1/85 (H3N2) – "Miss", or of highly virulent A/PR/8/34 (H1N1) – "PR8" (both human isolates). Negative control mice (non-infected) were given PBS. *In vivo* infectivity of these viruses is characterized as follows: the infectious dose causing 50% lethality of infected BALB/c mice, i.e. 1 LD_{50}, of the virus Miss is represented by 5,000 pfu, while that of PR8 virus is only 65 pfu (*5*). Differential leukocyte counts were done by microscopic examination (using immersion oil) of blood smears stained by the May-Grunwald solution (10 min.), followed by the Giemsa-Romanowski solution (15 min). These results were compared with differential white blood cell counts from negative control mice (Table 1). Differential white blood cell counts were calculated as a percentage of each kind of white blood cells.

Blood cell analysis of infected mice was performed on 0, 2, 4, and 7 days post infection (p.i.). Mice were infected with 0.1 or 0.4 LD_{50} of the moderately virulent virus Miss or with 0.1 or 0.4 LD_{50} of the highly virulent virus PR8. We found that the infection with 0.4 LD_{50} of the virus Miss led to a 3-fold increase in the neutrophil count on day 2 p.i. with subsequent slow decrease until day 7 p.i. (Fig. 1a) and returned to the normal level on day 10 p.i. (data not shown). In contrast, the proportion of lymphocytes dropped by 18% on day 2 p.i. and then gradually rose nearing the normal level. Other leukocyte subpopulations did not show any marked changes during the period between days 2 and 7 p.i. The infection with a lower dose (0.1 LD_{50}) of the Miss virus revealed similar changes in monitored blood cell parameters: increase of neutrophil count by 8% and decrease of lymphocyte count by 8% on day 2 p.i. (Fig.1a). Thus, it can be assumed that the infection of mice with IAV Miss caused lymphopenia, and, at the same time, an increase in the percentage of neutrophils in the peripheral blood. The extent of these changes is dependent on the infection dose. We also followed the proportion of peripheral blood cells after the infection with the virus PR8, which is more virulent than the strain Miss. Following the infection with 0.4 LD_{50}, the differential leukocyte counts showed that the number of lymphocytes dropped by 15.6% on day 2 p.i. – from 87.6% in non-infected mice to 72% in the infected animals (Fig. 1b). In next days, the proportion of lymphocytes in

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Abbreviations: IAV(s) = influenza A virus(es); p.i. = post infection

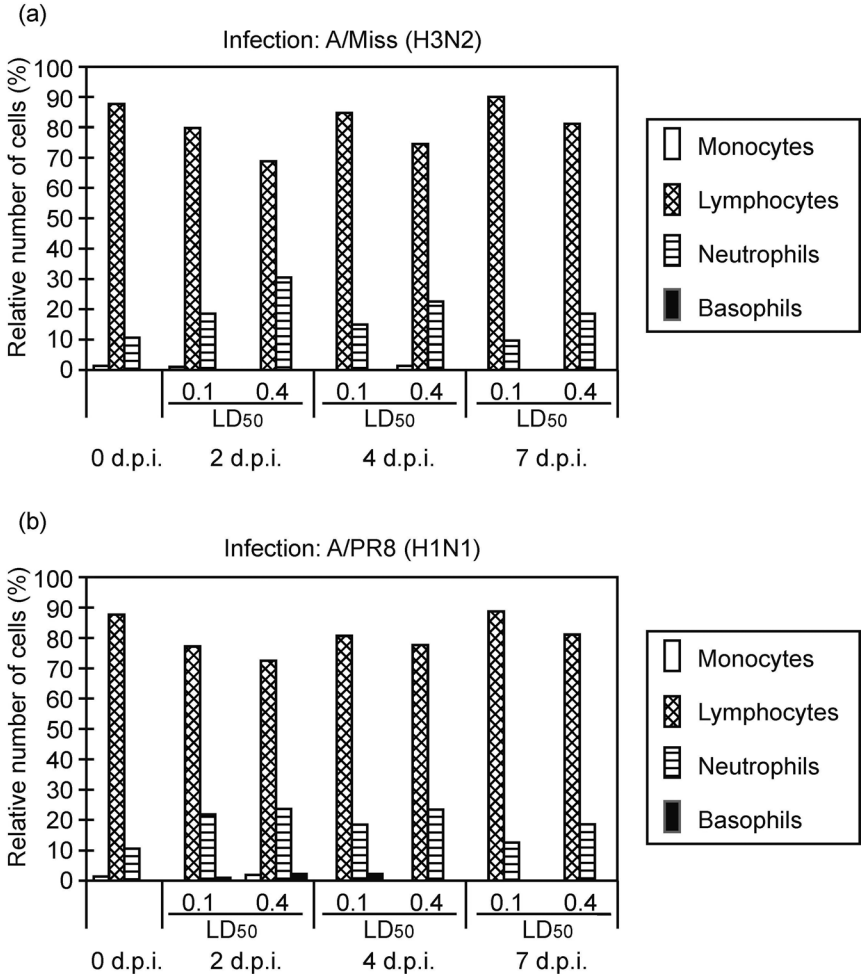

Fig . 1

Proportions of mouse leukocyte counts after IAV infection with A/Mississippi/1/85 (H3N2) (a) or A/PR/8/34 (H1N1) (b)

Leukocytes values (%) were determined from peripheral blood.

infected mice gradually increased to 77% on day 4 p.i. and 78% on day 7 p.i. At the same time, the neutrophil counts have repeatedly shown a reciprocal picture – a significant increase (more than two-fold compared with uninfected animals) on day 2 p.i., followed by a decrease on day 4 and 7 p.i. (Fig. 1b). This is similar to the neutrophil counts after infection with the strain Mississippi. Again, no other group of white blood cells displayed any significant changes. When mice were infected with a lower dose – 0.1 LD_{50} – of PR8 virus, the level of lymphocytes was decreased on day 2 and 4 p.i., but returned to the normal control value on day 7 p.i. The neutrophil counts were increased on days 2 and 4 post infection, but they reached normal level on day 7 p.i.

In conclusion, the changes in differential leukocyte count caused by the infection with the human IAV were clearly dose-dependent, i.e. the changes were milder upon the infection with a lower virus dose than upon the infection with a higher virus dose. However, they did not depend on the virulence of the virus used for infection.

Table 1. Proportions of the white cell types in blood of non-infected mice

Cell type	Relative number of cells in peripheral blood (%)
Monocytes	1.6 ± 1.36
Lymphocytes	87.6 ± 3.09
Neutrophils	10.8 ± 3.24
Bazophils	0.0 ± 0.00
Eozinophils	0.0 ± 0.00
Atypic cells	0.0 ± 0.00
Total	100.0 ± 6.94

Relative numbers of cells were calculated from a total number of leukocytes (100%) 11,360 ± 788 per 1 μl of mouse blood (n = 5).

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In all experiments presented in this paper, animals were treated according to the European Union standards and the fundamental ethical principles including animal welfare requirements were respected.

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