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"Rosettes" formation during mousepox in H-2^d mice: new insight into cellular and molecular mechanism of infectious ectromelia pathogenesis*

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Introduction and Aim. Antigen-specific activation of T cells is a key event in the successful induction of an adoptive immune response. Interactions between T cells and antigen presenting cells (APC) in structures called "rosettes" serve as an initial activation of CD4$^+$ T cells and are the presumed site of mutual recognition between T cells subsets *via* linked epitope (1, 2). It takes part in immune response or in the maintenance of self-tolerance, depending on the specificity and lineage of the T cells and the differentiation of the APC involved. Because the presentation of foreign antigen is a key event in the immune response, our studies were focused on the phenotype of APC (DC, Mø, B cells) and their quantitative relationship with effector cells (NK, T CD8$^+$/CD4$^+$, and B). Studies were performed with splenocytes isolated from BALB/c mice at different time p.i. with ectromelia (mousepox) virus (ECTV) (3, 4).

Materials and Methods. BALB/c (H-2^d) mice were infected with 10^4 PFU/mouse with highly pathogenic Moscow strain of ECTV (ECTV-MOS) into the hind footpads (total vol. 20µl/foot). Spleen and inguinal draining lymph nodes (DLN) were isolated at 2-3, 7, 14 and 21 d.p.i. For cell cluster preparation isolated organs were mechanically disaggregated by passing through stainless steel sieves (4). Then cells were fixed with 2% PFA, washed with PBS and labeled with fluorochrome conjugated Ab - for direct IF assay anti-ECTV-MOS-FITC (pAb), anti-CD3$^+$-PE (mAb), and anti-Pan-NK-PE (mAb) were used. Labeled cells were analyzed in fluorescence microscope (Olympus BX60), confocal microscope (Olympus IX81 FV1000), and scanning electron microscopy (JEM 1200 EX [JEOL Co.] combined with ASID 10 scanning attachment).

Results. Our studies showed that the number of effector cells clustered around each APC ranged from 3 to 9 (in spleen and DLN at 2 and 7 d.p.i.). At 2 d.p.i. average percentage of CD3$^+$ cells and NK cells in clusters obtained from spleen was 20% in both cases. After 7 d.p.i. the percentage of CD3$^+$ T cells increased up to 27%, but the percentage of NK cells decreased to 5%.

Conclusion. Preliminary data suggest involvement of NK and CD3$^+$ T cells in clusters formation with APC in early stages of infection, however the percentage of NK cells in "rosettes", which are the major component of innate antivirus response, decreased by the time during infection. Further investigations must be conducted to describe the role of B cells not only in clusters formation, but also in presentation of ECTV-MOS antigen(s) to the arms of the immune system.

References

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* *Grant nr 2 PO5A 050 30 from the Ministry of Science and Higher Education in Warsaw and WAU - SGGW grant nr 50402340017 [both for MN*]. E-mail: marek_niemialtowski@sggw.pl; [a] *PhD student*