7. Analysis of the molecular clock in CD4+ T cells and its relevance for the functional circadian rhythm of CD4+ T cells

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A number of immunological functions in CD4+ T cells are dependent on the circadian rhythm as we and others could previously demonstrate. Little is known about the underlying mechanisms. One possibility could be the circadian rhythm of the molecular clock in T cells. The molecular clock is known to control the circadian rhythm in the brain and several peripheral organs. To address this question we analyzed the expression of five clock genes (Bmal1, Per2, Cry1, Rev-erbα, Dbp), the production of cytokines and the CD40L expression in CD4+ T cells from human volunteers. A total of 15 healthy young men were examined under defined conditions over 24 h in the sleep lab. Venous blood was drawn periodically every 3 h, CD4+ T cells were isolated. T cells were split: one fraction was used for the investigation of clock gene expression and the second fraction was polyclonally stimulated and analyzed applying FACS. We found that on average ~32% of polyclonally stimulated highly purified CD4+ T cells express CD40L at 15:00 h whereas only ~2% of the CD4+ T cells express CD40L at 6:00 h. Additionally there is also a strong rhythm for the production of INF-g. Furthermore, we have preliminary data demonstrating the rhythmic expression of the core clock genes Bmal1and Cry1 in CD4+ T cells. These findings demonstrate that highly purified CD4+ T cells have a strong functional circadian rhythm and that a possible underlying mechanism could be the molecular clock.

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8. Local catecholamine production and synovial inflammation: New target for arthritis therapy?

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It is well demonstrated that norepinephrine can influence the immune response [1]. We already demonstrated that in RA synovial tissue there are cells producing catecholamines. To study the role of local catecholamine production in the inflammatory process in arthritis. Synovial samples were obtained from 21OA and 10RA patients who underwent knee joint replacement. Synovial cells were cultured with reserpine (10-6 to 10-8 M), OR486 (10-5 or 10-6 M) or medium (control) for 24 h. TNF, IL-8 and IL-6 were determined by Luminex and ELISA. For *in vivo* experiments, collagen type-II arthritis in DBA-1J mice was elicited. Thirty-one days after arthritis induction, mice were injected with 300 mg/kg reserpine or with NaCl (control) in the dorsum of one hind paw. Arthritis score was daily evaluated until day 47. The blockade of catecholamine by reserpine 10-6 M caused a strong reduction of TNF both in OA and RA. IL-6 is significantly lower in reserpine-treated cells of OA, whereas there is a reduction of IL-8 production only in reserpine-treated RA cells. The effects on cytokines were even stronger if reserpine was used in combination with OR486, an inhibitor of catecholamine degradation. Also *in vivo* caused reserpine a strong anti-inflammatory effect. In fact, 16 days after reserpine treatment the clinical score was lower in reserpine-treated mice compared to controls. We hypothesize that the local production of catecholamines in the synovial tissue plays a crucial role in the inflammatory process in arthritis. Therefore, modulation of catecholamine release by reserpine could be useful in the treatment of this pathology.

References

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9. Germinal centre innervation of bovine and human tonsils related to prion diseases

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In regard to BSE and vCJD, prion tropism for lymphoid tissues is different even if the infectious strain and the way of inoculation are identical. During vCJD, the agent crosses the digestive barrier and multiplies in lymphoid organs, before reaching the brain. Indeed, in vCJD, it accumulates in the ileum, tonsils, spleen and appendix of infected individuals. In contrast, in cattle, the BSE agent has a low affinity for lymphoid tissues and mainly accumulates in the nervous system. So, it appears that, at least in the case of BSE and vCJD, host properties can influence the accumulation of the infectious agent in lymphoid organs. We analyzed by confocal microscopy the mucosal innervation and the interface between nerve fibers and FDC in bovine and human tonsils using a panel of anti-bodies. Two categories of bovines (calves less than 12 months old and bovines older than 24 months) and two categories of humans (patients less than 5 years old and patients older than 25 years) were studied and has been compared to results observed in other organs like in lymph nodes and spleen and in other species like in mouse and sheep. Lymphoid tissue innervation is a dynamic process depending of animal species. In bovine and human species, ways of innervation by-passing germinal centers could be postulated: nerve fibers are widely distributed in antigen/cell traffic area: the lamina propria, the interfollicular zone and the lymphoepithelial area. We pointed out that, only in tonsils of bovines older than 24 months, nerve fibers are observed to be in contact with FDC. In contrast, in human tonsils, no nerve fibers established contacts with FDC, whatever the age. Innervation of germinal centers is an age-dependent dynamic process in bovines. The weak innervation of the lymphoid organs could thus be a rate-limiting step to neuroinvasion in humans. This species difference could influence the way of neuroinvasion and thus, the susceptibility of bovines and humans to the BSE agent.

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10. Destruction of brainstem noradrenergic neurons affects splenic cytokine production

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