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POLARIZED NEUTROPHILS IN HEPATOCELLULAR CARCINOMA FOSTER IMMUNE PRIVILEGE AND DISEASE PROGRESSION VIA PD-L1

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Background and Aims: Neutrophils are the most abundant circulating leukocytes in humans. Recently, dual roles of tumor-associated neutrophils (TANs) within the multiple step paradigm of cancer have been recognized. Although there is the evidence for the existence of N1 (antitumoral) and N2 (protumoral) TANs, similar as M1 and M2 macrophage polarization, it also need to clarify neutrophil effector molecules, such as phenotypic molecular markers, which will contribute to further insights and will be crucial for therapeutical applications in cancer pathologies.
Methods: Peripheral blood and intratumor neutrophils from 110 HCC patients assessed by flow cytometry. Immunocytochemistry were used to analyze the distribution and clinical relevance of neutrophils in 192 HCC patients. For functional analysis, human neutrophils are separated from by flow cytometry sorting and then co-cultured with CD4+/CD8+ effector T cells and tumor-specific antigen inducing CTLs for 48 h in vitro. Finally, in vivo analysis, the frequencies and function of neutrophils in peripheral blood. spleen and tumor are evaluated by flow cytometry.
Results: We show here that the expression of programmed death ligand 1(PD-L1), which interacts with PD-1, is increased in neutrophils from HCC patients compared with healthy controls and chronic hepatitis B patients. The expression of PD-L1 was significantly up-regulated on neutrophils after exposure to tumor supernatant from hepatoma cells, and IL-1, IL-6, TNF-αand TGF-βis largely responsible for this up-regulation of PD-L1. Tumor environmental factors induce early transient expression of PD-L1 on neutrophils in tumor bearing mice. The PD-L1+ neutrophils effectively suppressed the proliferation of CD4+/CD8+ effector T cells and tumor-specific T cell immunity, the effect could be reversed by blocking PD-L1 on those neutrophils in vitro. Tumor-bearing increased the frequencies of neutrophils in peripheral blood (mainly G2 population), bone marrow (mainly G3 population), and spleen (mainly G1 population). The infiltrating neutrophils in tumor showed high expression of PD-L1 and infiltrating CD3+ T cells showed high expression of PD-1, compared with peripheral blood and spleen in tumor-bearing mice.
Conclusions: These data suggest that polarized neutrophils in peripheral blood and intratumor of hepatocellular carcinoma with the high expression of PD-L1 may contribute to maintenance of immunosuppression and foster immune privilege.

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CHARACTERISING THE IMMUNE STATUS OF HBV-SPECIFIC CD4+ AND CD8+ T-CELLS PRODUCING IL-17 IN PATIENTS WITH CHRONIC HEPATITIS B (CHB) VIRUS INFECTION

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Background and Aims: Virus specific CD4+ and CD8+T-cells are essential in the control of HBV infection and their functions are tightly regulated by immune homeostatic control mechanisms, such as the programmed death (PD1) pathway, Tim3 and CD244. These pathways maintain the equilibrium between efficient control of viral replication and unnecessary inflammatory/immunopathological damage. Recent studies have described a unique subset of T-cells which produce IL-17. Preliminary studies suggest that IL-17-producing T-cells maybe involved in inflammation and liver damage, but largely the role of HBV-specific IL-17 producing CD4+(Th17) and CD8+(Tc17) T-cells during chronic HBV infection remains elusive. Moreover, the impact of immunoregulatory signatures on these T-cells are unknown. The aim of this study was to characterise the role and immune status of virus-specific Th17 and Tc17 cells in CHB patients.
Methods: Peripheral blood mononuclear cells were collected from 10 treatment naïve HBeAg+ CHB patients and 10 healthy controls. PBMC's were stimulated with recombinant HBcAg/HBsAg and PMA/Ionomycin. The frequency of total and virus-specific CD4+ and CD8+ T-cell producing IL-17/IFNg and the expression of T-cell immunoregulatory molecules PD1, Tim3 and CD244 was analysed by flow cytometry.
Results: Total number of CD8+ T-cells producing IL-17 was not different between chronic HBV patients and healthy controls. However, HBV-specific Tc17 cells were significantly higher in CHB-patients compared to controls ($p = 0.007$). Total Th17 were also higher in CHB-patients compared to controls ($p = 0.03$); however, the difference was more pronounced in HBV-specific CD4+ Th-17 ($p = 0.003$). Upon analysis of the immune-homeostasis signatures we found a higher expression of PD1 and CD244 on HBV-specific CD4+ and CD8+ T-cells producing IFNg ($p < 0.001$ and $p = 0.026$ respectively) in CHB patients. No expression of Tim3 was found on these cells. However, HBV-specific Th17 cells in the CHB patients did not express PD1 or CD244 but had levels of Tim3 significantly lower than healthy controls ($p = 0.027$).
Conclusions: This study reveals the involvement of virus-specific Th17 and Tc17 in the pathogenesis of chronic HBV infection. Interestingly, we observe differential patterns of immunoregulatory signatures operational within the populations of virus-specific T-cells producing IFNg and IL-17 which may influence their role in HBV disease.

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THE ABCS OF VIRAL HEPATITIS – DEFINING BIOMARKER SIGNATURES FOR ACUTE VIRAL HEPATITIS

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Background: Viral hepatitis is the leading cause of liver disease worldwide and can be caused by several agents, including Hepatitis A, B or C virus. Recent studies of the host response to infection has advanced the development of tools that allow for assessment of biomarker signatures to infection. We harnessed this approach to define unique signatures during acute viral hepatitis caused by HAV, HBV or HCV.
Methods: We performed multi-analyte profiling (MAP) measuring the concentrations of 196 analytes in the serum of acute Hepatitis A, Hepatitis B, or Hepatitis C infected individuals, as compared to healthy controls. Patients were recruited as part of a hospital based surveillance program in two "fever hospitals" specialized in infectious diseases in Cairo, Egypt. Acute infection was established using a combination of serological and PCR based assays.