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IMMUNOLOGY ABSTRACTS

Workshops

Workshop: Myeloid Cell Development

W01.001

Dysregulated hematopoietic stem and progenitor cell activity promotes IL-23-driven chronic intestinal inflammation

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Purpose/Objective: In IL-23-dependent colitis there is excessive accumulation of short-lived neutrophils and inflammatory monocytes in the intestine. It is not known whether this reflects changes in mature cell populations or whether the IL-23-driven colitogenic T cell programme regulates upstream hematopoietic stem and progenitor cells (HSPC).
Materials and methods: By analysing HSPC regulation in several mouse models of colitis, we investigated if hematopoieisis is dysregulated during chronic intestinal inflammation.
Results: Here we show dysregulation of hematopoiesis in colitis that is controlled by IL-23-driven inflammatory cytokines. Firstly there is an IFN-γ-dependent accumulation of proliferating hematopoietic stem cells (HSC) in the bone marrow (BM) and spleen. Secondly the hematopoiesis is strongly skewed towards granulocyte-monocyte progenitor (GMP) production at the expense of erythroid and lymphoid progenitors. Extramedullary myelopoiesis was also evident during intestinal inflammation and GM-CSF blockade reduced the accumulation of splenic and colonic GMPs. Importantly, adoptive transfer of peripheral GMPs during ongoing colitis exacerbated disease.
Conclusions: These data newly identify HSPCs as a major target of the IL-23-driven inflammatory axis suggesting new therapeutic strategies for the treatment of inflammatory bowel disease.

W01.002

Fate mapping of murine monocytes and macrophages exploiting CX3CR1 promoter activity

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Purpose/Objective: Mononuclear Phagocytes comprise a myeloid immune cell compartment including monocytes, macrophages and dendritic cells that collectively critically contribute to the maintenance of tissue integrity, as well as innate and adaptive immune defence. Emerging evidence for labour division among the mononuclear phagocyte subsets indicate that their manipulation could bear considerable therapeutic potential. However, specific ontogenies of individual populations and the overall functional organisation of the cellular network remain incompletely understood.
Materials and methods: Here, we report a fate mapping study of the murine mononuclear phagocyte compartment using mice that harbour genes encoding conditional or constitutive active Cre recombinases in their CX_3CR1 loci. Side-by-side comparison of CX_3CR1^{Cre} and CX_3CR1^{CreER} mice crossed to respective reporter animals, as well as CX_3CR1^{gfp} mice, combined with BrdU pulsing experiments and the analysis of CCR2-deficient mixed BM chimeras, provided critical insights into monocyte dynamics.
Results: In CX_3CR1^{Cre}:YFP mice and $CX_3CR1^{CreER:YFP}$, CX_3CR1 − tissue macrophages including liver Kupffer cells, lung alveolar, splenic and peritoneal macrophages were YFP+ and YFP-, respectively. Blood Ly6C- monocytes are 30% YFP-labelled in $CX_3CR1^{CreER:YFP}$ mice, and have half-life of approximately 2 days. BrdU pulse-labeling, adoptive transfer experiments, and analysis of the ontogeny of $Ly6C^-$ monocytes in $CCR2^{-/-}$ mice, strongly indicate that $Ly6C^-$ monocytes originate from $Ly6C^+$ moncoyte.
Conclusions: We demonstrate that major tissue resident macrophage populations are established prior to birth and maintain themselves subsequently during adulthood independent of replenishment by monocytes. Furthermore, we dissect the interrelationship of monocyte subsets and provide cumulative evidence that the inherently ephemeral $Ly6C^+$ monocytes form in steady state an obligatory precursor intermediate for the generation of potentially more long-lived $Ly6C^-$ monocytes whose life span is critical determined by presence of $Ly6C^+$ monocytes.

W01.003

Distinct bone marrow-derived and tissue resident macrophage-lineages proliferate at key stages during inflammation

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Purpose/Objective: The general paradigm is that monocytes are recruited to an inflammatory lesion where they terminally differentiate into macrophages. However, recent studies show that tissue resident macrophages can be derived from distinct lineages and are capable of self-renewal by proliferation. This can occur independently of peripherally recruited monocytes. Our objective was to analyse macrophage phenotype during inflammation and determine whether peripherally derived macrophages could proliferate.
Materials and methods: Monocytes and macrophages populations were purified and total RNA was subjected to Affymetrix microarray analysis. Cell cycle analysis of distinct macrophage populations was performed by 9-colour flow cytometry. Cellular origins were investigated using partial bone-marrow chimeras. IL-4Rα deficient mice were used in conjunction with IL-4 and M-CSF neutralising antibodies to investigate mechanisms of proliferation.
Results: Our data shows that proliferation of bone marrow-derived

Workshop: Lymphomas

W64.001

Immunoglobulin gene repertoires in stomach-associated lymphoid tissues — from gastritis to stomach lymphomas

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Purpose/Objective: Chronic gastritis is a common disorder characterized by chronic inflammation of the gastric mucosa. This chronic inflammation may be due to autoimmune responses or various foreign agents, such as bacterial infection, most frequently with *Helicobacter pylori.* The main health risk associated with chronic gastritis is development of gastric cancer. A strong association exists between gastritis, *H. pylori* and gastric low grade mucosa-associated lymphoid tissue (MALT) lymphoma, which in some cases further transforms into diffuse large B cell lymphoma (DLBCL). However, gastric DLBCL can also be initiated *de novo*. The mechanisms underlying transformation into the different types of DLBCL, and the differences between them, are not completely understood.
Materials and methods: Important information regarding clonal evolution forces (antigen affinity-driven selection and diversification) operating on B cells during chronic inflammation and lymphomagenesis can be revealed by sequence analysis of Ig variable region genes using Ig gene tree analysis and mutation pattern characterization. Towards this goal, we employed experimental and bioinformatical methods to reveal B cell clonal dynamics in samples from patients suffering from gastritis, gastric MALT lymphoma, or DLBCL with and without a chronic gastritis background.
Conclusions: The Gastritis of unknown reason (GNHP) is a condition with a very diverse repertoire, as diverse as that of lymph nodes. DLBCL are highly diverse*the transforming event can occur in any clone. However, there is a support for the assumption that chronic inflammation increases the chance for transformation. The positive selection towards replacement mutations raises the question of whether DLBCL clones need a functioning BCR or not.

W64.002

TNFRSF13B/TACI and TNFRSF13C/BAFFR in B cell chronic lymphocytic leukemia (B-CLL)

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Purpose/Objective: Recent studies indicate that TACI and BAFFR might participate in the survival of B-CLL cells, protecting them from apoptosis through NFkB activation. Considering that anti-TACI and anti-BAFFR therapeutic approaches are still available, the aim of this study was to determine the expression of *TNFRSF13B/*TACI and *TNFRSF13C/*BAFFR on B-CLL cells and their contribution to the phenotype and the prognosis of the disease.
Materials and methods: Peripheral blood and/or bone marrow from 104 patients with B-CLL (M/F: 59/45, mean age: 68.5 years) were examined. Thirty patients with low-grade NHL and 145 healthy individuals, age and sex matched, were used as controls. Complete hematologic, serologic and flow cytometric (FC) analyses were performed, including in B-CLL the most powerful prognostic factors (ZAP-70, CD38 and IgH mutational status). The mRNA and protein levels of *TNFRSF13B* and *TNFRSF13C* were estimated by qRT-PCR and flow cytometry (TACI clone 1a1, Abcam & BAFFR clone 11C1, Biolegend), respectively. BAFF and APRIL serum levels were estimated by ELISA. B-CLL cells exhibiting high or absent TACI, were stimulated by PKW, BAFF and APRIL and the apoptosis status was estimated by flow cytometry using an Annexin V-FITC/7-AAD (7-AAD) kit.
Results: B-CLL patients displayed significantly lower expression of TACI (mean ± SD: 13.4 ± 22%) compared to both NHL patients (36.8 ± 29%) and healthy controls (27.6 ± 14.2%), and lower MFI of BAFFR (6.71 ± 2.9) compared to healthy controls (10.7 ± 5.9). Interestingly, serum BAFF was absent in the majority of B-CLL patients. Additionally, B-CLL cells with low or absent TACI expression were more susceptible to apoptosis *in vitro* after stimulation with all mitogens, but *TNFRSF13B*/TACI expression was not significantly associated with the presence of autoimmunity, hypogammaglobulinemia, or monoclonal gammapathy, the infection risk and the abovementioned prognostic factors of B-CLL.
Conclusions: TACI expression is low in the majority of B-CLL patients, it is not associated with disease prognosis, and is accompanied by very low or absent serum BAFF. These findings should be taken into account in the case of anti-TACI and anti-BAFFR therapeutic approaches.

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W64.003

Preclinical study of a glycoengineered anti-human CD20 antibody in a murine model of primary cerebral B-cell lymphoma

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Purpose/Objective: Primary cerebral lymphomas (PCL), related to the systemic diffuse large B-cell lymphoma family, are highly aggressive tumors, with poor prognosis and any specific therapy. Despite good results obtained with high dose chemotherapy, many patients relapse and new therapeutic strategies are needed. PCL are characterized by the presence of $CD20^+$ lymphomatous B-cells and as such are eligible for therapy with anti human CD20 antibodies.
Materials and methods: In this study, we evaluated the efficiency of Ublituximab, a promising glycoengineered anti-hCD20 monoclonal antibody that displays a high affinity for human FcgRIIIa (CD16) receptors. We used a murine lymphomatous B cell line transfected with the human CD20 gene to generate a syngeneic murine model of PCL. Tumor cells were injected in the right striatum and treated 7 days later with one intratumoral or intrathecal injection of Ublituximab.
Results: After a single therapeutic injection of Ublituximab, a strong anti-tumor response was noted against lymphoma B cells. This was linked to an inhibition of tumor growth and infiltration with $CD8^+$ T-cells, and a long term survival for up to 50% of the treated mice. Interestingly, surviving mice rechallenged in the contralateral striatum with lymphoma B cells expressing hCD20 do survive without any further treatment.
Conclusions: These *in vivo* results confirm the therapeutic potential of