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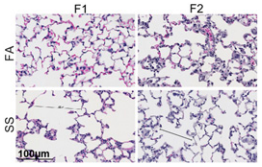
The Impact of Secondhand Smoke Exposure: It Runs in the Family

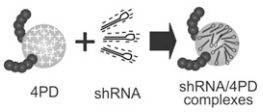


Although studies have linked gestational exposure to cigarette smoke (CS) with a high risk of allergic asthma (AA) and bronchopulmonary dysplasia (BPD), an increasing number of epidemiological studies suggest that gestational exposure to CS can also increase the risk of AA and BPD in grandchildren of women who smoke during pregnancy. In this issue, Singh et al. (p. 3815) demonstrated that exposure to environmental/secondhand/sidestream CS (SS) during pregnancy exacerbated allergen-induced Th2 responses and airway resistance both in first generation (F1) pups, which were gestationally exposed to SS, and second generation (F2) pups, which were not exposed to SS in utero, when compared with F1 and F2 pups from unexposed dams. Importantly, the transmission of this phenotype to the F2 generation was independent of F1 pup gender, and was exhibited by all members of the F2 progeny, indicating that inheritance of gestational SS-induced asthma involved epigenetic, rather than Mendelian genetic, transmission. Further examination of the lungs of 10-wk-old F1 and F2 pups from SS-treated dams demonstrated that gestational exposure to SS impaired alveolarization and angiogenesis, as evidenced by increased alveolar volumes and a downregulation of p65-NFκB, which is critical for angiogenesis in the developing lung. The asthma phenotype observed in F1 and F2 pups of SS-exposed dams also was associated with decreased HIF-1α levels in the airway and increases in both microRNA (miR)-16 and miR-221 in the lungs, which are known to decrease angiogenesis and increase apoptosis in various cell types. Conversely, miR-130a, which inhibits autophagy and stimulates angiogenesis, was significantly decreased in the lungs of F1 and F2 pups from SS-exposed dams. Together, these results highlight the potential for the transgenerational transmission of the effects of gestational CS exposure via epigenetic mechanisms regulating apoptosis and angiogenesis.

Cell Cycle Progress Is Gone in B Cells Lacking GON4L

In *Justy* mice, deficiency in the putative transcriptional regulator GON4L blocks B cell development at the transition from pre–pro-B cells to pro-B cells, but the function of GON4L remains poorly understood. To clarify the role of GON4L in B cell development, Barr et al. (p. 3978) analyzed cell cycle progression, proliferation, and mitotic gene expression in B cell precursors from *Justy* mice. These B cell precursors were more likely to be in the G1 phase of the cell cycle than their wild-type (WT) counterparts, suggesting cell cycle disruption was responsible for an observed reduction in pre–pro-B cell proliferation in GON4L-deficient cells. The IL-7 signaling pathway was intact in *Justy* cells and thus was not the source of the cell cycle defect; similarly, there were no deficiencies observed in the expression of the transcription factor PAX5 or in IgH recombination. Instead, reduced expression of genes encoding cyclins and E2F transcription factors in B cell progenitors from *Justy* mice suggested a mechanism for their cell cycle dysregulation. In support of these data from ex vivo analysis of bone marrow–derived B cell progenitors, comparison of WT and *Justy* B cell progenitors generated in culture also showed that the latter were blocked in differentiation at the pre–pro-B cell stage, despite intact IL-7 signaling. The cultured GON4L-deficient B cell progenitors demonstrated impaired DNA synthesis accompanied by DNA fragmentation and apoptosis; however, introduction of prosurvival factors could not rescue the defect in pro-B cell differentiation in these cells. Instead, enforced expression of cyclin D3, cyclin E1, or E2F2, which are all important for the G_1/S and/or S phases of the cell cycle, could significantly increase pro-B cell differentiation in B cell progenitors from *Justy* mice. Taken together, these data indicate an important role for GON4L at the G_1/S phase of the cell cycle in B cell progenitors that is key to pro-B cell differentiation.

Targeting Tumor-Localized Myeloid Cells



Tumor-educated myeloid cells (TEMCs) include many subtypes of innate immune cells that promote tumor progression, both directly by augmenting angiogenesis and metastasis and indirectly by suppressing antitumor immunity. Studies of these cells have been hampered by their intrinsic plasticity and the difficulty of recreating the tumor microenvironment in vitro. RNA interference (RNAi) techniques have the potential to modulate TEMC processes in vivo, but currently available procedures to deliver RNAi to these cells have numerous limitations. Zilio et al. (p. 4166) aimed to overcome many of these limitations through development of a nanoparticle, 4PD, that could efficiently target TEMCs in vivo. To accomplish this, a peptide targeting IL-4Rα, which is upregulated on myeloid cells following tumor exposure, was conjugated to fifth generation polyamidoamine dendrimers, and the resultant 4PD particles were confirmed to recognize myeloid-derived suppressor cells (MDSCs) and macrophages in tumors. The 4PD dendrimers were conjugated to the amphipathic molecule cardiolipin to reduce their immunogenicity and to short hairpin RNA (shRNA) targeting STAT3 or C/EBPβ. Treatment of tumor-bearing mice with the 4PD particles loaded with STAT3-specific shRNA reduced STAT3 expression in MDSCs and impaired their suppressive activity, confirming the utility of this system to deliver functional shRNA. Transfection could also be tracked in vivo by loading

the 4PD/shRNA complexes with brUTP, allowing the fate of transfected cells to be monitored. Both STAT3 and C/EBPβ could be targeted at once, and this treatment could augment the polarization of M1-type macrophages in tumors, increase the numbers of tumor-reactive T cells, and delay tumor growth. Further analysis using the microRNA miR142-3p revealed that 4PD particles could also be used to supply functional microRNA to tumor-associated myeloid cells, and miR142-3p delivery could synergize with adoptive cell therapy to inhibit tumor progression. This system shows promise for in vivo analysis of genes and microRNAs in TEMCs through specific targeting, which should advance the study of these cells and their involvement in tumor progression.

RGC-32 Regulates Th17 Responses

The response gene to complement (RGC)-32 can stimulate or suppress cell division, depending on the cell type and the environment in which it is expressed. RGC-32 is induced by TGF-β in cells including fibroblasts and astrocytes, and its expression is upregulated in the brains of patients with multiple sclerosis (MS). Because Th17 cell responses are promoted by TGF-β and play a proinflammatory role in autoimmune diseases such as MS, Rus et al. (p. 3869) investigated the potential involvement of RGC-32 in Th17 differentiation and in the experimental autoimmune encephalomyelitis (EAE) model of MS. RGC-32 was upregulated by TGF-β in mouse $CD4^+$ T cells and was expressed at a higher level in Th17 cells than in other $CD4^+$ T cell subtypes. Analysis of $CD4^+$ T cells from RGC-$32^{-/-}$ mice indicated that this molecule was important for the differentiation of Th17, but not Th1, Th2, or regulatory T cells. Although previous data demonstrated increases in IL-2 production and proliferation by RGC-$32^{-/-}$ $CD4^+$ T cells under Th0 conditions, these effects were not seen under Th17 conditions and did not seem to contribute to the observed defect in Th17 polarization. Instead, RGC-32 deficiency appeared to impair Th17 differentiation at the priming stage by inhibiting multiple pathways, resulting in impaired activation of transcription factors including SMAD2, BATF, IRF4, and RORγt. In an EAE model, RGC-$32^{-/-}$ mice developed significantly less severe disease than wild-type mice through a T cell-intrinsic mechanism, and this reduced disease was associated with reductions in the CNS of $CD4^+$IL-17^+ T cells and, surprisingly, $CD4^+$GM-CSF^+ T cells. This study defines an important role for RGC-32 in Th17 differentiation and suggests that it could serve as a therapeutic target for Th17-mediated autoimmune diseases.