RHEUMATOID ARTHRITIS

Synergy of environmental and genetic risk in RA

“AhR ligands exacerbate collagen-induced arthritis in SE-positive mice”

The risk of rheumatoid arthritis (RA) is affected by a combination of environmental factors, such as exposure to cigarette smoke, and genetic susceptibility that is mostly mapped to the *HLA-DRB1* locus, but functional links between these two risk areas have not been defined. New research in *PNAS* indicates that the shared epitope (SE) in HLA-DR β chains synergizes functionally with aryl hydrocarbon receptor (AhR) activation to drive nuclear factor- κ B (NF- κ B) signalling and thereby exacerbate inflammatory arthritis in mice.

“It has been known for some time that exposure to cigarette smoke works synergistically with the presence of SE-coding genes in promoting RA risk,” notes Joseph Holoshitz, corresponding author of the new study. However, the mechanisms behind this relationship have until now been unclear.

AhR is a ligand-activated transcription factor that responds to a diverse array of natural and synthetic environmental and endogenous ligands, including polycyclic aromatic hydrocarbons that are present in cigarette smoke. AhR is also important in adaptive immune responses as it contributes to the activation and differentiation of

T helper 17 (T_H17) cells, which are cells that are central to the pathogenesis of autoimmune diseases, including RA.

Holoshitz and colleagues began investigating the synergy of AhR signalling with SE in RA having noticed that many of the pathways activated by AhR ligation are identical to those activated by SE, a five-amino-acid sequence within the HLA-DR β chain that his lab has studied extensively.

“The SE functions as a ligand that interacts with the cell surface receptor calreticulin to transduce pro-inflammatory signalling,” explains Holoshitz. This concept, established from previous work in their lab, is a crucial piece of the puzzle, as calreticulin is known to activate NF- κ B, which in turn can drive osteoclastogenesis and the polarization of T_H17 cells, both of which contribute to RA.

To prove the existence of a statistically significant synergy between AhR and SE signalling, and thereby reveal a possible molecular mechanism underlying RA risk, the researchers used 2-way ANOVA to compare two different background strains of transgenic mice: mice that express SE-positive *HLA-DRB1**04:01 alleles and mice that express SE-negative *HLA-DRB1**04:02 alleles.

First, they added an AhR signalling agonist (either 6-formylindolo[3,2-b]carbazole (FICZ) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)) to bone marrow cells (BMCs) from these mice *ex vivo*. AhR enhancement of receptor activator of NF- κ B ligand (RANKL)-induced osteoclastogenesis (measured by TRAP staining) was greater in BMCs from SE-positive mice than in cells from SE-negative mice.

Synergy of AhR and SE signals was identified in response to costimulation of RAW cells with FICZ or TCDD plus a synthetic SE ligand (65-79*0401). In these experiments, the degree of osteoclastogenesis and NF- κ B activation, and induction of the inflammatory arthritis-associated proteins C-C motif chemokine 2 (CCL2) and cathepsin K, was greater with combination stimulation than the sum of the responses to individual stimuli.

The combination of AhR and SE signalling also resulted in an increase in polarization of T_H17 cells in cocultures of dendritic cells with naive T cells.

In sum, these stimulation-based cellular assays indicate a common intersection of AhR and SE-induced signalling pathways at the point of NF- κ B activation that drives RA pathogenesis. Indeed, the authors finally showed *in vivo* that AhR ligands exacerbate collagen-induced arthritis in SE-positive mice. These mice developed disease earlier and had more severe bone erosions and disease severity (as measured by radiography and micro-CT) than SE-negative mice.

Although these new data do not include a direct molecular link between cigarette smoke and RA susceptibility, joining the dots of the now extensive literature relating to cigarette smoke, AhR activation and SE signalling, plus what is known about the associated downstream signalling pathways, provides an enticing mechanism to account for a substantial portion of the environmental and genetic risk associated with RA.

To find direct evidence for these links, Holoshitz says he next wants to examine this interaction in mice exposed to cigarette smoke, plus he plans to begin translational studies using human cells.

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