Journal highlight: Impact of eosinophil-peroxidase (EPX) deficiency on eosinophil structure and function in mouse airways[1]

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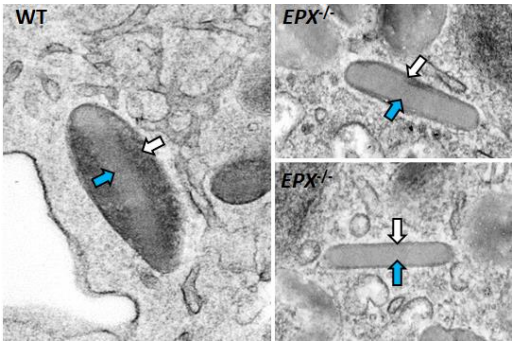
Eosinophil peroxidase (EPX or previously abbreviated EPO) is a cationic granule protein uniquely expressed in eosinophils. EPX is able to generate oxidant species that contribute to the antibacterial activity of eosinophils against gram negative bacteria. Even though EPX is a major constituent of both mouse and human eosinophil granules, previous studies employing EPX deficient mice found no significant differences in eosinophil structure, function or recruitment to the lungs and airways. In this article, Percopo et al. examine eosinophils from EPX$^{-/-}$ mice following intranasal challenge with the aeroallergen *Alternaria alternata*, circumventing the use of adjuvants, which potentiate Th2 response. Using this approach, they discovered reduced numbers of eosinophils in the lungs, spleen and bone marrow of EPX$^{-/-}$ mice compared to wild type controls. Flow cytometric analysis showed that these eosinophils express less Siglec-F and lower levels of TLR4+ eosinophils were found in the lungs of EPX$^{-/-}$ challenged mice. Moreover, by using ELISA to measure cytokine levels released by isolated eosinophils from the lungs of challenged mice, the authors discovered lower levels of the pro-survival cytokine IL-3 being made in EPX$^{-/-}$ mice. Even though eosinophils from EPX$^{-/-}$ mice appear normal at the light microscopic level, their transmission electron micrographs display profound granule abnormalities and reduced volume of granule matrix.

Discovering significant alterations in the structure and cytokine content of EPX$^{-/-}$ eosinophils, the authors tested whether they have an impaired ability to clear gram negative *Haemophilis influenzae*. Surprisingly, bacterial clearance of these eosinophils was not impaired. Percopo et al. are the first to show significant structural alterations in eosinophils of EPX$^{-/-}$ mice that, regardless of their maintained bacterial clearance capacity, may impact other functions.



Transmission electron micrographs of eosinophils from wild-type (WT) and eosinophil peroxidase gene-deleted (EPX$^{-/-}$) mice. Blue-colored arrows indicate the granule core, and open arrows indicate the granule matrix.

Question and answer with senior author Dr. Helene Rosenberg.

Q: In this article you focus on evaluating EPX deficiency in mouse eosinophils. What prompted you to look into this specific disorder, which is very rare in humans?

A: This is indeed a very rare disorder of humans, and one that has been considered to be totally benign, meaning, the patients are found either on routine check-up or secondary to some other, unrelated chief complaint. This disorder was first identified by Ben-Zion Presentey and colleagues who were performing routine blood work on a Yemenite Jewish family in Israel[2]; two more cohorts have since been identified, one in Northern Italy[3], and another from Japan[4]. There may be more individuals with this deficiency, but no one is really on the look-out for this. However, we were very intrigued by observations initially made by the Lee laboratories using mice devoid of major basic protein (MBP-1) and then *both* granule proteins eosinophil peroxidase (EPX) and major basic protein (MBP). The former mice (MBP-1$^{-/-}$) do have eosinophils (e.g., they can be detected by flow) but they do not stain red, and they are very dysmorphic[5]. Most intriguing, if you add EPX deficiency on top of that (MBP-1$^{-/-}$EPX$^{-/-}$) the mice simply don't make any eosinophils[6]. We helped to characterize these latter mice with our bone marrow culture system[7], and showed that this was in fact an endogenous issue; bone marrow progenitors without these two granule proteins are simply incapable of undergoing maturation either *in vivo* or in culture. So, what about EPX? The Lee laboratories had already generated these mice[8], as above, and we had them as part of the original collaboration; given the tools we had in hand, we thought that they were worth a second look. We examined cell surface receptors, cytokine contents, and various functionalities, which are described in the manuscript[1]. It remains astonishing that, given the substantially reduced granule matrix size, the cytokine content was virtually identical, save for reduced concentrations of IL-3 among the eosinophils from the EPX$^{-/-}$ mice. Nonetheless, given what we now know about human eosinophil cytokine variability (see below), I would love to have the chance to evaluate the cytokine contents of eosinophils from human subjects with this disorder.

Q: In your career you have consistently pushed the boundaries of mouse eosinophil research. What in your opinion are the biggest challenges of translating mouse eosinophil data to human clinically relevant findings?

A: This is a really good question that all of us need to think about all the time. Mice are not humans (and humans are not mice) and eosinophils in particular, while similar in nature and overall morphology, have distinct receptors, components and responses. As but one of several examples, human eosinophils are chock-full of a protein known as galectin-10, or Charcot-Leyden crystal protein, while this protein is completely absent in mice. Likewise, mouse eosinophils do not have direct orthologs of EDN or ECP (see below), and Siglec-F/Siglec-8 are functional (as opposed to direct, or sequence-specific) paralogs. As such, one must always ask oneself "What are the critical features of the response that I am examining" and "Are these critical features replicated in this mouse model"? This is true of virtually anything one might choose to model in mice. The important part is designing the experiment and asking questions in a way that provides you with useful information.

Q: In order to study eosinophil recruitment and function of EPX$^{-/-}$ mice you used *Alternaria alternata* as an aeroallergen. There is a growing number of allergen-challenge models being used in preclinical studies. This might on the one hand make comparisons of results across studies more difficult, but on the other hand provides additional information. How do you view this issue?

A: We have been working *Alternaria alternata* as an aeroallergen here and in other studies, and we utilize a strategy that results in eosinophil recruitment to the lungs that does not involve intraperitoneal sensitization. In our lab, we are actively moving towards more physiologic aeroallergens and administration strategies, and *Alternaria alternata* is an indoor and outdoor allergen that has been associated with the development of allergic asthma in human subjects. There certainly are quite a few allergen challenge models (house dust mite, fungal allergen, acute *vs.* chronic) and while it will make direct comparison confusing for a time, this will ultimately help all of us to understand which methodology (all? any?) most clearly replicates the allergic inflammatory responses observed in (some / all / any) human subjects.

Q: Did you ever have an "eureka" moment connected to eosinophil research in the lab, and if yes, what was it?

A: OK, this is really back in the “olden days” so let me try to put this in context – PCR was in its infancy, no sequencing of any entire genomes yet. I had identified cDNA clones for human EDN and human ECP, collectively, the eosinophil RNase A ribonucleases, for my post-doctoral work in Dr. Steven Ackerman’s lab (first eosinophil genes, from cDNA libraries[9,10] and as part of my initial studies at the outset here at NIAID, my assistant, Kim Dyer and I sought to identify the mouse orthologs as the first steps toward *in vivo* studies. This proved to be impossible by standard methodologies of the day, which were all based a high degree of sequence similarity (anyone remember Southern blots?). However, somewhere along the line it occurred to us that impossible may really mean interesting. Via the primate consortium at Duke University, as well as cell lines from ATCC, we were able to collect DNA samples from multiple Great Apes, Old World and New World Monkeys. From that point, we were able to explore and to establish the absolutely astonishing evolutionary pathway taken not only by the eosinophil ribonucleases EDN and ECP, still the most rapidly evolving coding sequences known among primates[11], which provided us with important hypotheses regarding function, but we ultimately elucidated most of the extensive human / primate RNase A ribonuclease gene family, which at that time was totally unknown.

P.S winds up of course that finding the mouse ribonucleases was not impossible, just harder than one might have anticipated. Larson and colleagues from the Lee laboratories ultimately identified two mouse eosinophil-associated RNases (mEars) from scratch, starting from protein sequence[12]. Then Jianzhi Zhang in my group went ahead and identified multiple mEars (12 or so) and showed that the rodent eosinophil RNase A family ribonucleases also had an interesting evolutionary story of its own, having diverged from one another by rapid gene sorting and positive selection, a methodology employed by the T cell receptor family, among others[13].

Q: Eosinophil research is quickly extending beyond the traditional ‘allergy borders’ all the way to cancer, obesity and other research areas. In which direction is your lab headed?

A: We are very intrigued with the concept of eosinophil heterogeneity, and the factors that may have subtle (or not so subtle!) impact on their development both before and even after they are released into circulation. We have recently found that the cytokine content of human eosinophils can vary dramatically from one individual to another. This is intriguing because, by the principals of the “LIAR” (Local Immunity And/or Remodeling/Repair) hypothesis, if eosinophils in humans are also responsible for local immunomodulation, regeneration and repair, then differences in cytokine content may have an impact on one’s ability to defend against infection, respond to medication, and/or recover from disease. We do not yet know why this is the case, but we are looking to understand how this happens, so that we can then design meaningful mouse studies to explore mechanism.

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