Medical Immunology Campus Erlangen

An Interdisciplinary Center of the Friedrich-Alexander-Universität Erlangen-Nürnberg

Newsletter Summer 2020

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Scientific Highlights

FRIEDRICH-ALEXANDER UNIVERSITÄT ERLANGEN-NÜRNBERG

EDITORIAL

Dear colleagues and friends,

During the past 7 months, COVID-19 has been an extraordinary challenge for the entire society, but also for the scientific community. Besides the restrictions in public and private life, the pandemic had a negative impact on the economic development, the school and university education, and the usual forms of scientific exchange such as national and international conferences. A new form of German *Angst* spread at the universities and led to temporary shutdowns of research laboratories that were not working on COVID-19. On the other hand, the pandemic also provided a cornucopia of new chances and opportunities: it taught many people the basic principles of hygiene, raised new interest of politicians in infectious diseases, and motivated the federal and state ministries to pour out unprecedented amounts of research money, frequently without the usual scientific and administrative hurdles. Furthermore, as scientists, we have witnessed an explosion of publications on SARS-CoV2 with extremely short periods from submission to acceptance, even in high-ranking journals that normally tend to torment researchers with several rounds of meticulous peer reviews. For infectious disease experts, immunologists and epidemiologists, it was quite an amazing experience to see that the new virus triggered interdisciplinary research activities that have never, or at least not to the same intensity, been undertaken in the context of other important respiratory infections. Finally, we all became used to holding and attending ZOOM conferences, which saved us a lot of travelling time and even allowed us working furtively on other things in our offices. The future will show whether the political decisions, the financial adventures, and the scientific endeavours will pay off beyond the containment of the COVID-19 pandemic.

Fortunately, COVID-19 did not bring the scientific developments in Erlangen to a standstill. A few weeks ago, the DFG Grants Committee approved the proposal of Hans-Martin Jäck, Diana Dudziak and Udo Gaipl for a new Research Training Group "FAIR" on adaptive immunity. Congratulations to the coordinators and all the project leaders for this fantastic achievement! Another highlight was the successful acquisition of 40 million € for a new research building termed CITABLE *(Center for Immunotherapy, Biophysics and Digital Medicine)* that will be located between the TRC1 and the Medical Clinics. This is primarily a merit of Markus Neurath, who not only created the idea and coined the name, but also was responsible for the application to the *Joint Science Conference of the Federal Government and the Governments of the Federal States.*

As of today, the guest lectures in the forthcoming winter term will be primarily held as ZOOM seminars. The annual *Joachim Kalden Lecture* had to be postponed until late spring/early summer 2021.

Have a nice vacation break, and please remember the key rule for this SOMMER (**so**cial distance, **u**se **m**ask, **m**anual hygiene, **e**ntry from **r**isk areas requires testing or quarantine).

Christian Bogdan

Prof. Christian Bogdan

Chairman of The Medical Immunology Campus Erlangen

SCIENTIFIC HIGHLIGHTS

IFN-λ induced intestinal epithelial necroptosis drives Crohn's Disease-like intestinal inflammation

Interferon λ promotes Paneth cell death via STAT1 signaling in mice and is increased in inflamed ileal tissues of patients with Crohn's disease.

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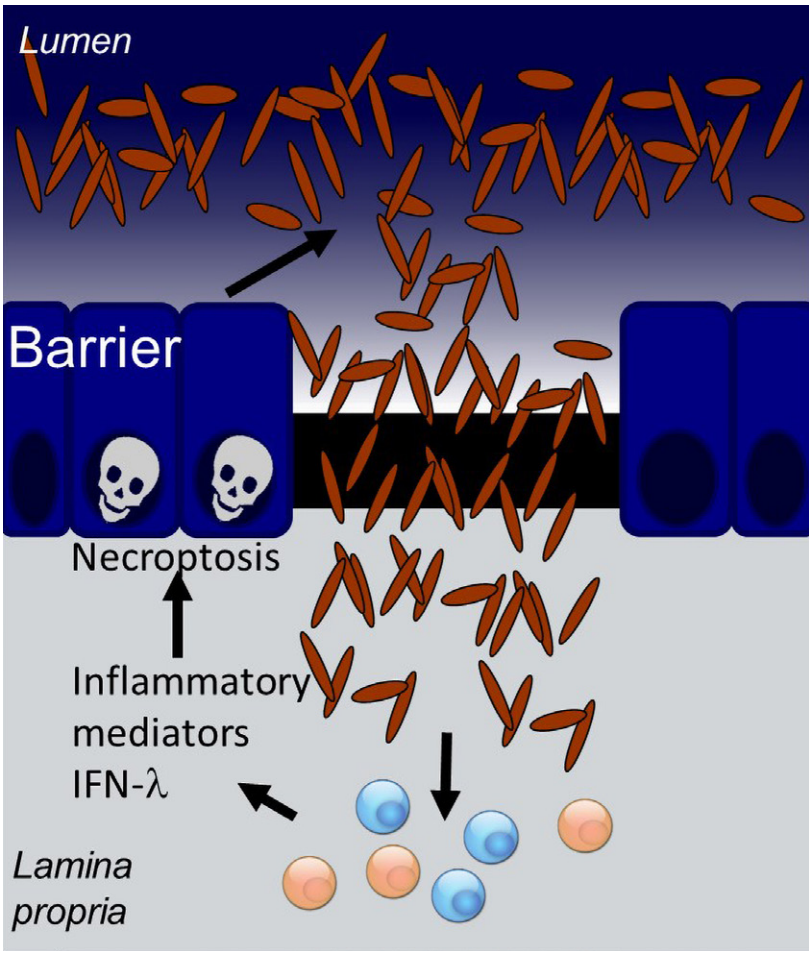
Despite considerable efforts, the pathophysiology of Crohn´s disease (CD) is still not well understood. There is, however, overwhelming evidence that the pathogenesis of CD is characterized by an exaggerated immune reaction, presumably directed against commensal enteric bacteria in genetically susceptible hosts.

Our work over the years support a model according to which cytokines derived from activated immune cells in the gut can affect the homeostasis of intestinal epithelial cells (IECs). Alterations in IEC barrier functions such as excessive apoptosis and necroptosis in turn promote the translocation of luminal antigens into the bowel wall causing aberrant and excessive immune and cytokine responses. Barrier dysfunction, microbial invasion and inflammatory IEC death (leading to more barrier dysfunction) constitute a vicious cycle, which prevents resolution of inflammation and drives chronic intestinal inflammation.

Our current work demonstrates a key role for interferon lambda (IFN-λ) in driving IEC death, barrier dysfunction and persistent inflammation. We demonstrate elevated levels of IFN-λ in gut samples of CD patients. The expression of IFN-λ in mice resulted in an almost complete depletion of Paneth cells, cells with crucial functions in controlling microbial access to the body. On a molecular level, we identified that IFN-λ signaling via STAT1 caused Paneth cell death. We further demonstrate that Paneth cell death is driven by MLKL-mediated necroptosis in a caspase-8 regulated manner. Collectively, our results implicate a pathophysiological role for IFN-λ during ileal inflammation by compromising Paneth cell homeostasis and antimicrobial defense.

Gunther C., Ruder B., Stolzer I., Dorner H., He G.W., Chiriac M.T., Aden K., Strigli A., Bittel M., Zeissig S., Rosenstiel P., Atreya R., Neurath M.F., Wirtz S. and Becker C. 2019. Interferon Lambda Promotes Paneth Cell Death Via STAT1 Signaling in Mice and Is Increased in Inflamed Ileal Tissues of Patients With Crohn's Disease. Gastroenterology. 157.5:1310-1322 e13



SCIENTIFIC HIGHLIGHTS

Neutrophils drive formation and growth of gallstones

Neutrophil extracellular traps initiate gallstone formation

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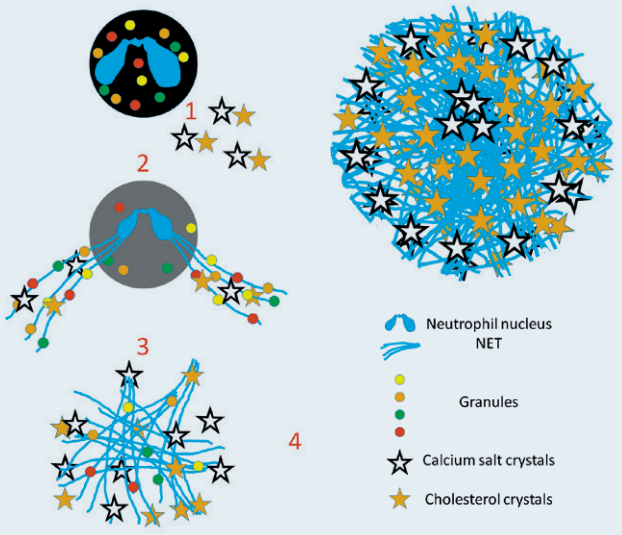
Gallstones are very common. Around six million Germans suffer from gallstones, which often cause extremely painful colic. As a less frequent complication, gallstone disease may precipitate life-threatening inflammation of the abdominal cavity. Stone ailments very often require surgical interventions. Surprisingly, little is known about the pathogenesis and growth of gallstones.

We found that biliary "sludge" and human gallstones contain NET components (including citrullinated histones). Citrullination of histones is a marker for NET formation and is performed by enzymes referred to as peptidyl arginine deiminases (PADI), especially by the neutrophil-borne PADI4. To further investigate, we constructed an *in vitro* model for the "building" of artificial gallstones. We employed small gallstone gravel and known gallstone ingredients as a starting point (nidus). Importantly, the formation of larger artificial stones required the addition of neutrophils and some kind of mechanical movements. Employing high-resolution microscopy, we found that DNA from NETs glued the crystals together and that this was crucial for the formation of larger "stones" in a layering process.

Next, we focused on the cell-biological pathways that could be pharmacological targets to prevent stone formation. As crystals are established to be potent inducers of NET formation, we investigated inhibitors of NET synthesis. Indeed, either genetic ablation of the oxidative burst or of PADI4 resulted in a strongly reduced formation of diet-driven murine gallstones. Similar results were obtained by pharmacological inhibition of PADI4 or by a classical blocker of beta1-adrenergic signalling, known to stun neutrophils preventing their migration to the tissue.

Together, our data suggest that neutrophils initiate the development and growth of gallstones. This process turned out to be driven by reactive oxygen species and by calcium-dependent activation of the citrullination machinery. Crystals from calcium salts and cholesterol are the preformed building blocks of gallstones. They activate patrolling neutrophils to form NETs and are glued together when these mate and form extended aggregates.

Munoz L.E., Boeltz S., Bilyy R., Schauer C., Mahajan A., Widulin N., Gruneboom A., Herrmann I., Boada E., Rauh M., Krenn V., Biermann M.H.C., Podolska M.J., Hahn J., Knopf J., Maueroder C., Paryzhak S., Dumych T., Zhao Y., Neurath M.F., Hoffmann M.H., Fuchs T.A., Leppkes M., Schett G. and Herrmann M. 2019. Neutrophil Extracellular Traps Initiate Gallstone Formation. Immunity. 51. 3: 443-450 e4



Model of gallstone formation

1 crystals of calcium salts and cholesterol meet neutrophils that patrol the ducts of exocrine glands, e.g. bile ducts.

2 this triggers neutrophils to externalize their chromatin decorated with a plethora of nuclear, cytoplasmic and granular proteins (NET formation).

3 these so called neutrophil extracellular traps immobilize the crystals and form small microliths in the biliary ducts including the gallbladder.

4 With time, these microliths grow and form full-size gallstones by appositional addition of further crystals and NETs.

SCIENTIFIC HIGHLIGHTS

Prenylated RhoA as a regulator of T cell homing to the colon and intestinal immune homeostasis

Inhibiting PGGT1B disrupts function of RhoA, resulting in T-cell expression of integrin α4β7 and development of colitis in mice

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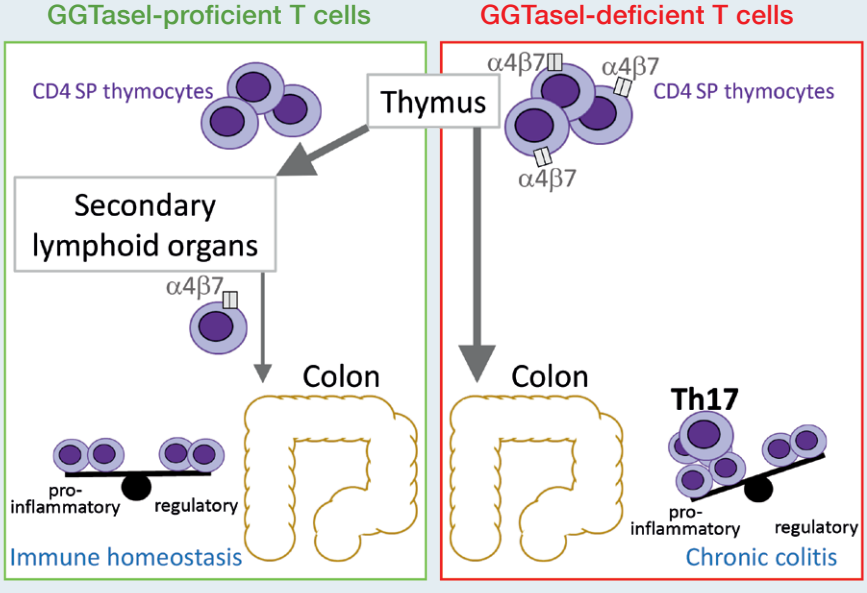
Inflammatory bowel diseases (IBD) are characterized by chronically remitting flares of severe intestinal inflammation. Locally accumulated and overwhelmingly activated pro-inflammatory T cells within the intestinal mucosa of affected patients represent an important therapeutic target in IBD. However, the molecular mechanisms underlying the pathological T cell trafficking and effector function in IBD still remain incompletely defined.

Our work identified a crucial role of GGTasel-catalyzed prenylation for the regulation of intestinal T cell responses. Analyzing mice with a T-cell-specific deletion of *Pggt1b* (gene encoding for GGTasel) (*Pggt1b*$^{\Delta CD4}$ mice), we observed that the absence of GTTasel in T cells resulted in a spontaneous and age-dependent development of chronic colitis. Of particular interest regarding the clinical relevance of these findings, a significantly decreased expression of GGTasel could also be detected in T cells infiltrating the intestinal lamina propria of IBD patients. Mechanistically, thymus-egressing GGTasel-deficient CD4$^+$ thymocytes showed an elevated expression of the gut homing marker α4β7 and were prone to a selective migration into the colonic mucosa. Moreover, colon-infiltrating CD4$^+$ T cells in *Pggt1b*$^{\Delta CD4}$ mice could be characterized by increased frequencies of RORC-expressing and IL17A-producing inflammatory effector T cells. Aiming at the identification of the key target protein of prenylation in this context, our data pointed to the small GTPase RhoA as a potential linker between impaired prenylation in T cells and the development of chronic colitis and, thus, suggest a targeted modulation of RhoA prenylation or signaling as a promising strategy for IBD therapy.

Lopez-Posadas R., Fastancz P., Martinez-Sanchez L.D.C., Panteleev-Ivlev J., Thonn V., Kisseleva T., Becker L.S., Schulz-Kuhnt A., Zundler S., Wirtz S., Atreya R., Carle B., Friedrich O., Schurmann S., Waldner M.J., Neufert C., Brakebusch C.H., Bergo M.O., Neurath M.F. and Atreya I. 2019. Inhibiting PGGT1B Disrupts Function of RHOA, Resulting in T-cell Expression of Integrin alpha4beta7 and Development of Colitis in Mice. Gastroenterology. 157. 5: 1293-1309



The absence of the prenylation-catalyzing enzyme GTTasel in T cells results in a preferential homing of thymus-egressing CD4 single positive (SP) thymocytes to the colon, an up-regulated expression of pro-inflammatory cytokines in colon infiltrating CD4$^+$ T cells and, finally, in the development of chronic colitis.

SCIENTIFIC HIGHLIGHTS

New insights into IgA function

IgA subclasses have different effector functions associated with distinct glycosylation profiles

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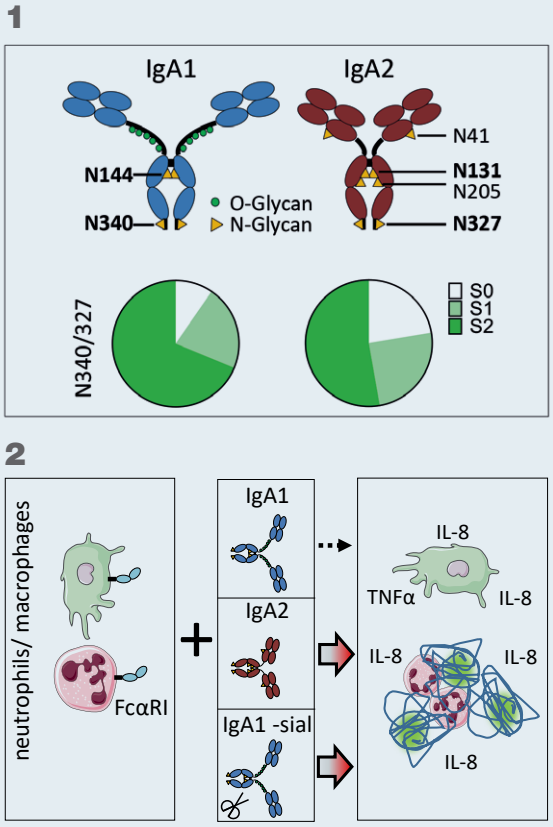
Immunoglobulin A (IgA) is the most abundantly produced antibody in the human body. In contrast to dimeric IgA that is rapidly secreted into mucosal tissues, monomeric IgA stays in the serum where it represents the second most frequent antibody class. Serum IgA induces inflammatory and anti-inflammatory effects via the Fcα-receptor I (FcαRI) and seems to be involved in several autoimmune diseases. However, the regulation of serum IgA effector function is not well defined.

Our work revealed that the two human IgA subclasses (IgA1 and IgA2) act differently on neutrophils and macrophages. While IgA2 induced a marked enhancement of neutrophil extracellular trap (NET) formation and IL-8 production, IgA1 did not show pronounced effects.

In depth glycosylation analysis using mass spectrometry revealed different glycosylation patterns of IgA1 and IgA2 at the two conserved glycosylation sites. Overall, IgA2 displayed lower degrees of sialylation, galactosylation and bisecting N-acetylglucosamine. The increased sialylation might be a reason for the non-inflammatory phenotype of IgA1, as cleavage of sialic acid, but also of the whole N-glycans increased the pro-inflammatory capacity of IgA1 to the level of IgA2.

Taken together, our data demonstrate that IgA effector functions depend on subclass and glycosylation. This finding likely is of relevance for autoimmune diseases because in patients with rheumatoid arthritis, disease-specific autoantibodies against citrullinated proteins (ACPA) display a shift toward the pro-inflammatory IgA2 subclass, which is associated with higher disease activity.



Regulation of IgA effector functions

1) IgA1 and IgA2 possess several N-linked glycosylation sites. Sites conserved between the two IgA subclasses are written in bold letters. Below, the portions of nonsialylated (=S0), monosialylated (=S1) and bisialylated (=S2) glycans are shown for the N-terminal glycosylation site.

2) IgA2 and desialylated IgA1 induce much stronger NET formation and IL-8 release than IgA1 after binding to FcαRI on neutrophils and macrophages.

Steffen U., Koeleman C.A., Sokolova M.V., Bang H., Kleyer A., Rech J., Unterweger H., Schicht M., Garreis F., Hahn J., Andes F.T., Hartmann F., Hahn M., Mahajan A., Paulsen F., Hoffmann M., Lochnit G., Munoz L.E., Wuhrer M., Falck D., Herrmann M. and Schett G. 2020.
IgA subclasses have different effector functions associated with distinct glycosylation profiles.

SCIENTIFIC HIGHLIGHTS

Fra-1 inhibiting arginase-1 in macrophages promotes arthritis

Transcription factor Fra-1 targets arginase-1 to enhance macrophage-mediated inflammation in arthritis

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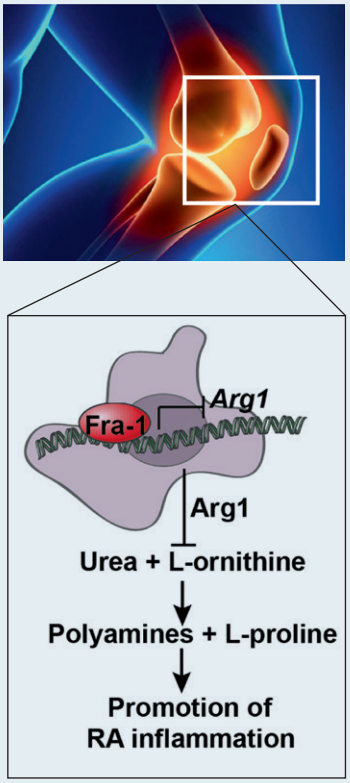
During rheumatoid arthritis (RA), characterized by synovial hyperplasia, cartilage degradation, and bone destruction, the proinflammatory macrophages are deeply involved in the induction phase, whereas antiinflammatory macrophages promote the resolution of inflammation. Fra-1 protein, a member of the AP-1 family, is a key transcriptional regulator for macrophage signaling. Thus, we explored its role in rheumatoid arthritis.

We found that Fra-1 expression is increased in synovial macrophages of patients with active RA when compared to patients in remission. Using *in vitro* macrophage stimulatory models and CHIP sequencing analyses, we identified arginase 1 (Arg1) as a target of Fra-1.

In several experimental mouse models, FRA1-deficient mice had decreased arthritis severity, including reduced immune cell infiltration and bone erosion. Mechanistically, the activity of ARG1 was increased in FRA1-deficient mice compared with wild-type controls. ARG1 catalyses the conversion of L-arginine to L-ornithine and has known immunoregulatory functions. Treatment of FRA1-defici-ent mice with an arginase inhibitor increased RA severity to the extent present in wild-type mice.

Finally, we investigated the effect of increasing arginase activity through L-arginine supplementation either at arthritis onset or at the peak of inflammation (representative of a therapeutic setting) to see that both approaches strongly reduced inflammation and bone degeneration.

Together, our data might provide a new avenue for therapeutic opportunities by altering macrophages responses from a pro-inflammatory to a pro-resolving state through the modulation of the L-arginine pathway.



Fra-1 in macrophages inhibits Arg1 expression and promotes RA inflammation

Hannemann N., Cao S., Eriksson D., Schnelzer A., Jordan J., Eberhardt M., Schleicher U., Rech J., Ramming A., Uebe S., Ekici A., Canete J.D., Chen X., Bauerle T., Vera J., Bogdan C., Schett G. and Bozec A. 2019. Transcription factor Fra-1 targets arginase-1 to enhance macrophage-mediated inflammation in arthritis. J Clin Invest. 129. 7: 2669-2684

Medical Immunology Campus

An Interdisciplinary Center of the Friedrich-Alexander-Universität Erlangen-Nürnberg

Publisher
Medical Immunology Campus Erlangen
An Interdisciplinary Center of the Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU)

www.mice.uni-erlangen.de

Conceptual Design and Editor
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