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Editorial

Editorial: **To resolve or not to resolve: Annexin A1 pushes resolution on track**

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Ground-breaking work during the last 10 years, by a relatively small number of laboratories worldwide, has detailed the mechanisms that are now collectively referred to as the area of resolution of inflammation. Characterization of specific endogenous pathways, which dampen the acute inflammatory response, has led to an appreciation of the fact that inflammation does not terminate spontaneously but rather, in a coordinated—in time and space—fashion. We are now aware of a group of mediators, ranging from short-lived lipids, autacoids, peptides, and proteins, which produce specific biological effects, all helping a proper induction of resolution of acute inflammation.

An important concept in resolution is that it is an active process [1]. Initially, endogenous anti-inflammatory mediators have been studied for their ability to inhibit cell trafficking (a hallmark of the inflammatory response), thus stopping the egress of blood-borne white cells from vessels to the tissue site of inflammation or infection. However, additional properties of these mediators soon emerged, including rapid removal of apoptotic cells by resident macrophages through the process of efferocytosis.

A specialized pool of macrophages appears during the resolution phase of acute inflammation [2]; these are the cells that mastermind the restoration of homeostasis, beginning with the removal of apoptotic leukocytes. Therefore, it is evident that a complex series of processes is required for resolution to operate in a swift and active fashion. Prior to efferocytosis, there is promotion of apoptosis (once again, an active phenomenon), entailing regulation of the lifespan of migrated immune cells, which is fundamental to avoid the propagation of the tissue damage that could ensue from a full-blown necrotic process [1].

For an organ or tissue affected by an inflammatory insult to return to its normal physiology, two other active processes must take place. The first one is priming of the adaptive immune system, such that any further encounter with the etiological insult can be dealt with rapidly. It is plausible that cell trafficking out of the site of inflammation, possibly via the lymphatic, must take place to activate the adaptive immune instruction in the LNs. Second is the orchestrated repair of the affected tissue, which requires activation of stromal cells (or mesenchymal stem cells), with differentiation to the specialized cell and production of ECM proteins to fully regain tissue functionality. This latter aspect has, so far, been investigated—and, in part, detailed—in the context of skin or gut epithelia, but it is most likely also to be relevant to compartments, such as the bronchial epithelium, the kidney mesothelium, and the joint cartilage.

Which are these mediators of resolution? Cortisol was the first hormone to exert crucial, protective actions and shown to be central for modulation of the host response to stress, from hemorrhage to trauma, infection, and metabolic stress to inflammation. In all cases, activation of the hypothalamus-pituitary-adrenal axis leads to cortisol release, which impedes the overshooting of the host reaction to stress [3]. It is not surprising, therefore, that the proresolving properties of glucocorticoids are the archetypal characteristics of a genuine proresolving mediator; examples include the control of leukocyte lifespan and the promotion of efferocytosis [1]. However, excess glucocorticoids often dampen, instead of activating, the adaptive immune response and delay tissue repair. It is therefore important that resolution programs are controlled and promoted by a plethora of mediators with largely similar, but not totally identical, biological properties. Short-lived lipids, such as lipoxins and resolvins, exert tight control on resolution [1, 4], and similar crucial functions are emerging for specific peptides and proteins with longer half-lives, such as galectins [5], chemerin peptides [6], and the α-melanocyte-stimulating hormone [7].

A combination of in vivo and in vitro assays is often used to elucidate the pathopharmacological features of specific effectors of resolution. Data emerging from in vitro assays will need to be validated in vivo. As an example, the proresolution mediator AnxA1 exerts

Abbreviations: AnxA1=Annexin A1, Bax=Bcl-2-associated X protein, Mcl-1=myeloid cell leukemia 1

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exquisite control over white blood cell egress outside postcapillary venules and promotes tissue repair [8], as demonstrated using in vivo and in vitro assays, including proof-of-concept validation with AnxA1 null mice (reviewed in ref. [9]). However, the ability of AnxA1 to promote neutrophil apoptosis and their removal by efferocytosis, reported by several laboratories, have hitherto only been demonstrated in vitro [10, 11].

In this issue of *JLB*, Vago et al. [12] fill this important gap in our knowledge by demonstrating the proapoptotic function of endogenous AnxA1 during an ongoing inflammatory reaction. Using a model of acute inflammation, which resolves naturally, the group of Sousa and Teixeira [12] makes use of elegant biochemical analyses, coupled with pharmacological tools, to conclude convincingly that exudate AnxA1 expression and its integrity are both of paramount importance in controlling the kinetics of resolution of inflammation (**Fig. 1**). The authors detail some of these mechanisms, including activation of proapoptotic pathways (e.g., caspase-3) and inhibition of survival signals (e.g., NF-κB). These data complement the signaling of lipoxin modulation of apoptosis investigated by Filep's group [14] and the promotion of phagocytosis of apoptotic cells by lipoxins and AnxA1-derived peptides, by Godson's lab [11].

These novel findings allow us to comment on a further degree of complexity or a new checkpoint, operative during an acute, self-limited inflammation. This is effected on the integrity of the AnxA1 protein, hence, the balance between proteolytic enzymes and antiproteases present in the inflammatory exudate. Which enzymes are responsible for AnxA1 proteolysis in the inflammatory milieu? Supported by data from studies in human asthma, it is accepted that the serine proteases elastase and proteinase-3, both released by extravasated neutrophils, are major proteolytic enzymes for AnxA1 [15]. Although not shown in this novel study, it is likely that these serine protease activities would peak during maximal inflammation, hence, in correspondence to the increased amount of cleaved AnxA1. Consistent with this conclusion and the importance of intact AnxA1 for actively promoting the resolution of pleurisy are the recent results with an Anx1 mutant, resistant to serine protease activity [16]. The data presented by Vago et al. [12] clearly demonstrate a fundamental, nonredundant role for endogenous AnxA1 in controlling the lifespan of migrated neutrophils; the authors study the intracellular pathways controlled by intact AnxA1 in relation to promotion of apoptosis, noting augmented Bax expression with associated reduction of survival pathways (e.g., Mcl-1; Fig. 1). The downstream, functional consequence of AnxA1 proteolysis on the dynamics within the inflammatory exudate is also a novel aspect of this study.

In summary, the novel study published in this issue of *JLB* completes a gap in our knowledge of the biology of the glucocorticoid-regulated protein

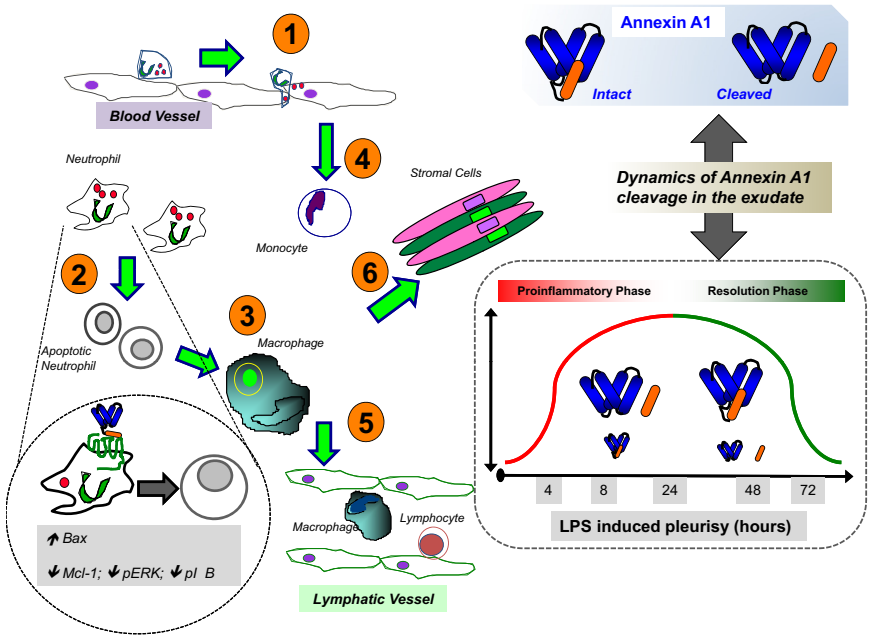

Figure 1. AnxA1 is the active drive behind the resolution of inflammation. Reported are the processes regulated by AnxA1—shown here with its schematic representation with the four repeats (70–80 aa each; in blue) and the unique N-terminal region (52 aa; in orange)—characteristics of resolution of acute inflammation. Leukocyte recruitment to the site of injury is a hallmark of the host inflammatory response and sets in motion a series of processes that are required to activate resolution, bringing about tissue repair and ultimately, regain of homeostasis. AnxA1 promotes several features of resolution by exerting potent inhibition on the leukocyte adhesion and extravasation cascade (feature 1) and promoting efferocytosis of apoptotic neutrophils by resident proresolution macrophages (feature 3). The new study of Vago et al. [12] demonstrates the unequivocal, nonredundant role of AnxA1 in promoting apoptosis of neutrophils (feature 2 of resolution in the figure). This occurs by activating Bax and caspase-3 cell death pathways with concomitant inhibition of the survival pathways (magnifying lens, bottom left). AnxA1 is also known to act as a costimulus toward T cell skewing (hence, kick-starting the adaptive immune response, possibly also, by favoring egress into the lymphatic system; feature 5) and to promote repair (feature 6). Vago et al. [12] add another mode for controlling the dynamics of resolution, that is, the cleavage of AnxA1. As schematized in the magnified cartouche at bottom right, proteolysis takes place in exudates, spanning from 4 to 24 h poststimulus, i.e. during the proinflammatory phase of pleurisy, which is then characterized by a majority of cleaved AnxA1, while intact AnxA1 predominates throughout the resolution phase (here, at the 48- and 72-h time-points; see data in ref. [12]. We could postulate that generation of cleaved products from the AnxA1 N-terminal region, in the early stages of the inflammatory reaction, can promote specific responses, including monocyte subendothelial space locomotion (feature 4), as shown recently in cell systems [13], but yet not proven in vivo. Collectively, exudate intact AnxA1 activates and coordinates all major processes required for a physiological resolution of inflammation, in this manner, controlling the outcome of experimental pleurisy. pERK/pIκBα, Phosphorylated ERK/IκBα.

AnxA1. The notion that the protein controls the lifespan of the neutrophil once extravasated to the site of inflammation [12] is of great importance and may have implications, not only for our appreciation of the dynamic processes involved in orchestrating resolution but also for approaches aimed at harnessing this wealth of knowledge to develop AnxA1 mimetics endowed with powerful, proresolving properties.

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REFERENCES

1. Serhan, C. N. (2011) The resolution of inflammation: the devil in the flask and in the details. *FASEB J.* **25,** 1441–1448.
2. Stables, M. J., Shah, S., Camon, E. B., Lovering, R. C., Newson, J., Bystrom, J., Farrow, S., Gilroy, D. W. (2011) Transcriptomic analyses of murine resolution-phase macrophages. *Blood* **118,** e192–e208.
3. Munck, A., Guyre, P. M., Holbrook, N. J. (1984) Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocrine Rev.* **5,** 25–44.
4. Serhan, C. N. (2007) Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu. Rev. Immunol.* **25,** 101–137.
5. Cooper, D., Iqbal, A. J., Gittens, B. R., Cervone, C., Perretti, M. (2012) The effect of galectins on leukocyte trafficking in inflammation: sweet or sour? *Ann. N. Y. Acad. Sci.*, Epub ahead of print.
6. Cash, J. L., Christian, A. R., Greaves, D. R. (2010) Chemerin peptides promote phagocytosis in a ChemR23- and Syk-dependent manner. *J. Immunol.* **184,** 5315–5324.
7. Muffley, L. A., Zhu, K. Q., Engrav, L. H., Gibran, N. S., Hocking, A. M. (2011) Spatial and temporal localization of the melanocortin 1 receptor and its ligand {α}-melanocyte-stimulating hormone during cutaneous wound repair. *J. Histochem. Cytochem.* **59,** 278–288.
8. Babbin, B. A., Laukoetter, M. G., Nava, P., Koch, S., Lee, W. Y., Capaldo, C. T., Peatman, E., Severson, E. A., Flower, R. J., Perretti, M., Parkos, C. A., Nusrat, A. (2008) Annexin A1 regulates intestinal mucosal injury, inflammation, and repair. *J. Immunol.* **181,** 5035–5044.
9. Perretti, M., D'Acquisto, F. (2009) Annexin A1 and glucocorticoids as effectors of the resolution of inflammation. *Nat. Rev. Immunol.* **9,** 62–70.
10. Solito, E., Kamal, A., Russo-Marie, F., Buckingham, J. C., Marullo, S., Perretti, M. (2003) A novel calcium-dependent proapoptotic effect of annexin 1 on human neutrophils. *FASEB J.* **17,** 1544–1546.
11. Scannell, M., Flanagan, M. B., deStefani, A., Wynne, K. J., Cagney, G., Godson, C., Maderna, P. (2007) Annexin-1 and peptide derivatives are released by apoptotic cells and stimulate phagocytosis of apoptotic neutrophils by macrophages. *J. Immunol.* **178,** 4595–4605.
12. Vago, J. P., Nougeira, C. R. C., Tavares, L. P., Soriani, F. M., Lopes, F., Russo, R. C., Pinho, V., Teixeira, M. M., Sousa, L. P. (2012) Annexin A1 modulates natural and glucocorticoid-induced resolution of inflammation by enhancing neutrophil apoptosis. *J. Leukoc. Biol.* **92,** 249–258.
13. Blume, K. E., Soeroes, S., Keppeler, H., Stevanovic, S., Kretschmer, D., Rautenberg, M., Wesselborg, S., Lauber, K. (2012) Cleavage of annexin A1 by ADAM10 during secondary necrosis generates a monocytic "find-me" signal. *J. Immunol.* **188,** 135–145.
14. El Kebir, D., Jozsef, L., Khreiss, T., Pan, W., Petasis, N. A., Serhan, C. N., Filep, J. G. (2007) Aspirin-triggered lipoxins override the apoptosis-delaying action of serum amyloid A in human neutrophils: a novel mechanism for resolution of inflammation. *J. Immunol.* **179,** 616–622.
15. Vong, L., D'Acquisto, F., Pederzoli-Ribeil, M., Lavagno, L., Flower, R. J., Witko-Sarsat, V., Perretti, M. (2007) Annexin 1 cleavage in activated neutrophils: a pivotal role for proteinase 3. *J. Biol. Chem.* **282,** 29998–30004.
16. Pederzoli-Ribeil, M., Maione, F., Cooper, D., Al-Kashi, A., Dalli, J., Perretti, M., D'Acquisto, F. (2010) Design and characterization of a cleavage-resistant Annexin A1 mutant to control inflammation in the microvasculature. *Blood* **116,** 4288–4296.

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Editorial: **Protean effects of IL-10 include skin self-defense**

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In this issue of the *Journal of Leukocyte Biology*, Takiishi et al. [1] have detailed their relatively surprising discovery that donor IL-10 production was essential for the acceptance of syngeneic murine skin isografts. Keratinocytes, which have long been known to produce IL-10, particularly under certain conditions, such as UV irradiation [2], appeared to be the primary cellular source for the protective IL-10. As such, IL-10, which can protect against rejection when overexpressed in a solid organ allograft [3], also plays a role in syngeneic graft acceptance. The mechanism of allograft rejection is largely T cell-mediated, whereas the study by Takiishi et al. [1] indicates that the mechanism of syngeneic graft failure likely involves infiltration of "innate" cells into an area of surgical tissue injury. Specifically, the investigators found that infiltration of neutrophils into the syngeneic graft was curtailed by graft production of IL-10 (see **Fig. 1A**). Importantly, host IL-10 production was less important than isograft IL-10 production, indicating that high local cytokine concentrations in the isograft microenvironment were critical for graft protection. Further studies using host cells

Abbreviations: PEG-IL-10=pegylated IL-10, Tc1=T-cytotoxic 1

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