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EDITORIAL

Earth: The planet of the annexins☆

La Tierra: el planeta de las anexinas

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Annexins are a protein superfamily evolutionarily retained and widely extended in nature.[1] Hundreds of these proteins are virtually present in living beings from the five kingdoms, from complex multicellular eukaryotes to unicellular eukaryotes and different forms of prokaryotes. They are classified into five categories: annexin A (in vertebrates), annexin B (in invertebrates), annexin C (in fungi), annexin D (in plants) and annexin E (in prokaryotes). The first annexins were discovered at the end of the 1970's and the beginning of the 1980's by groups who were researching scaffolding-involved proteins that were bound to membranes and other proteins. The two essential characteristics that define annexins are their ability to bind to Ca2+-dependent phospholipids and the presence of their C-terminal domain end characteristic of some 70 aminoacid repeat sequences. The N-terminal domain, which is less preserved, is thought to be the component which evolves faster and is responsible for the variety of functions of these proteins. Their function is closely linked to their capacity to dynamically and reversibly bind to cellular membranes, which they stabilize, promoting interactions in cytoplasmic membrane and Ca2+-regulated endocytic and exocytic processes. Although most annexins are cytoplasmic, there are nuclear forms and some which are relevant at cardiovascular level located on the cellular surface area where they act as co-receptors or are secreted and circulate through the blood stream. Twelve annexins (Anx) have been identified in human beings with a highly varied tissue expression and distribution pattern, that is called AnxA1 to AnxA13 (to date no gene has been allocated to AnxA12). The fact that deficient animal models in some of them were perfectly viable suggested that at first they could be highly redundant. However, posterior investigation has revealed a high level of specialisation that we are only just beginning to understand.[2] Due to their great variety and versatility they become essential components of multiple biological processes, such as the organisation and repair of membranes, vesicular traffic and subcellular traffic, calcium metabolism, proliferation, differentiation, apoptosis, adhesion, migration and invasion. Their common involvement in several human pathologies has led to the coining of the term '''annexinopathies'' to refer to diseases where the function of annexin levels have been altered. In recent years a large

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number of publications has familiarized us with the annexins involved in highly prevalent diseases such as cardiovascular diseases, diabetes, obesity, autoimmune diseases, cancer and infections.[2–6]

In this issue of *Clin. Invest. Arterioscler.*, Méndez-Barbero et al.[7] present an exhaustive review of the biology of annexins which is mainly focused on their impact in inflammation and other key mechanisms in the vascular remodelling associated with atherosclerosis. Mention is also made of their vital role in cellular membranes and in vesicular traffic that converts them into essential elements in the control of intracellular cholesterol homeostatic. Several annexins, such as A1, A2, A6 and A8 have effectively been involved in the cellular transport of cholesterol through the endocytic pathway, especially at late endosome level.[8] Out of them all it is AnxA6 that appears to play the most important role in the control of cholesterol distribution in intracellular compartments. On regulating membrane cholesterol content the annexins interfere with SNARE (*Soluble N-ethylmaleimide-sensitive fusion-Attachment protein [SNAP] Receptors*) proteins, a group of membrane proteins which are cholesterol-dependent that aid the fusion and traffic of vesicles responsible for transporting the molecules that are required for cell functioning. The article reviews published findings from the last two decades on annexins and atherosclerosis, focusing mainly on annexins A1, A2, A3, A5 and A7. AnxA1 is notable as an anti-inflammatory molecule through glucocorticoids regulation and innate immune response activation. This annexin reduces the attachment of neutrophils to the vascular endothelium and increases apoptosis, whilst inducing recruitment and differentiation of monocytes to anti-inflammatory phenotypes, effects that would explain its antiatherogenic activity.[3] The expression of AnxA1 increases in carotid platelets of asymptomatic patients, and in experimental atherosclerosis models the administration of a peptide derived from this protein reduces the progression of atherosclerosis and stabilises the platelets, highlighting its role in atherosclerosis and its therapeutic potential. In fact, as our knowledge of AnxA1 increases so does the revelation of its multifaceted nature. AnxA1 has recently been related to the aggregation of extracellular vesicles and vascular calcification[9]; at platelet level thrombin signalling is suppressed, activation of the CIIbB3 receptor is reduced and through selective modification of determinant of the surface area (e.g. phosphatidylserene) it promotes the phagocytosis of the platelets through neutrophils, thus triggering active resolution.[10] All these mechanisms are important in atherotrombosis. The ''dialogue'' between the ABCA1 cholesterol transporter and AnxA1, the expression of which is regulated by ApoAI, could also contribute to its atheroprotector role.[11] Thus the results from a recent animal test and patient study suggest that AnxA1 regulates the lipid metabolism and improves the progression of diabetic nephropathy.[12] AnxA2 is unique in its interaction with a S100 protein (protein p11), thanks to which it acts as a plasminogen receptor and tissue activator of plasminogen (tPA), playing a relevant role in fibrinolysis[13] and modulating the thrombotic effect of tPA.5 These activities of AnxA2 suggest they could reduce the hypercoagulability of the blood and thereby modulate atherothrombosis. Furthermore, its expression in macrophages and smooth vascular muscles cells (SVMC) is linked with the migration of these in atherosclerosis and restenosis, and their interaction with C5 integrin with inflammatory response.[14] Last but not least is their interaction with the protein convertase subtilisin/kexin type NF-KB (PCSK9), which it inhibits, acting as an endogenous modulator, participating in one of the main regulation mechanisms of LDL circulating levels.[15] In the most highly studied annexin group and on which there is greater proof that links those with vascular biology AnxA5 also plays a part. This annexin is secreted and circulates in the blood stream. Its ability to bind to phosfatidylserine from the cellular membrane confers it with antithrombotic, antiapoptotic and anti-inflammatory properties and has made it an essential tool for analysing cell death and in molecular imaging studies, for detecting high risk platelets. AnxA5 regulates the polarisation of macrophages[16] and appears to intervene mainly in the initial phases of atherogenesis.[17] In different preclinical models the administration of a recombinant protein reduces adhesion of leukocytes and macrophages and has been able to reduce the development of atherosclerotic lesions and make them more stable. It also reduces inflammation and myocardial damage in models of ischemia-reperfusion injury.[18] In patients, the circulating levels of AnxA5 have been inversely related with the severity of coronary stenosis, for which the oxLDL/AnxA5 ratio appears to be a better marker than LDL circulating levels. These and other results in the Méndez-Barbero and col. Review make AnxA5 into one of the most interesting annexins as a biomarker and prospective treatment for atherosclerosis and its complications.

Although the annexins mentioned up until now are those that have been most credibly involved with some of the mechanisms involved at the onset, progression and clinical complication of atherosclerosis, others appear to also play a major role on a cardiovascular level. We should not fail to mention AnxA3 for its involvement in angiogenesis and because its silencing promotes the repair of myocardial infarction4 and salvages SVMC function in intracranial aneurisms.[19] Finally, the important role of AnxA7 in Ca2+ homeostasis is particularly noteworthy in cardiac conditions such as auricular fibrillation, ventricular tachycardia, arrhythmias and cardiac remodelling. Its inhibition also reduces the size of, atherosclerotic lesions and stabilizes them.[20] The abundance of experimental evidence supporting the potential of annexins as therapeutic targets is increasing day by day.

To sum up, in what is now over 40 years since the first annexins were described, we have learned much about the complex biology of these proteins, about their huge functional variety and their potential as therapeutic targets. This explains the huge interest these proteins have given rise to in the international scientific community. It is such that the advances in this field are reviewed and discussed periodically in specific congresses, such as the biennial International *Conference on Annexins*, which, following a forced absence due to COVID-19, will again be celebrated in Stockholm in June in 2022 and this will be its 11th edition. This series of conferences has been key to the dissemination and promotion of the intense biomedical research that has been undertaken around these proteins. The near future will undoubtedly favour us with surprising novelties on this singular family of proteins. We hope that the Méndez-Barbero

and col. review stimulates interest in annexins among the readers of *Clin. Invest. Arterioscler.* and in general among all those interested in this positively translational research.

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