REVIEW ARTICLE

THE COUNTER-REGULATION OF ATHEROGENESIS: A ROLE FOR INTERLEUKIN-33

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Summary: The recently recognized cytokine interleukin-33 and its receptor ST2 play a favorable role during atherogenesis by inducing a Th1→Th2 shift of the immune response. IL-33 also protects the failing human heart from harmful biomechanical forces which lead to cardiomyocyte hypertrophy and exaggerated interstitial fibrosis. IL-33 inevitably displays side effects common to other Th2 cytokines, the most grave of which is a predisposition to allergic reactions. IL-33 is a nuclear transcription factor of endothelial cells. As such, it is abundant in nonproliferating vessels. Its down-regulation is required for angiogenesis, which may be profitable in wound healing or deleterious in tumor growth.

Key words: *Interleukin-33; ST2 receptor; Th2 immune response; Atherosclerosis; Antibodies; Angiogenesis*

Introduction of interleukin-33

Interleukin (IL)-33 represents a newly recognized member of the IL-1 cytokine family, which further includes interleukin-1β and interleukin-18. In accord with both IL-1β and IL-18, interleukin-33 is endowed with a strong immunomodulatory capacity. Contrary to its associates, both of which are known to promote Th1-skewed immune reactions, IL-33:

1. starts off the production of Th2 cytokines, namely IL-5 and IL-13, and
2. increases the levels of plasma immunoglobulins.

The latter are elaborated by B-lymphocytes which have been activated by signals delivered by Th2-lymphocytes and their cytokines (32).

IL-33 has been shown to be the ligand for the orphan receptor ST2.

The ST2 gene encodes two isoforms of a protein:

1. **ST2L**, which represents the functionally active transmembrane form, and
2. **sST2**, which is a soluble form of the protein that can be detected in human plasma.

From the viewpoint of cardiology and cardiac surgery, the ST2 protein is active in cardiac myocytes and fibroblasts that are challenged with excessive biophysical forces, namely mechanical strain (29). Apart from ST2, cardiac fibroblasts under mechanical strain can concomitantly produce IL-33. Whether generated locally in an autocrine/paracrine manner or carried by the blood stream, the IL-33 ligand is bound by ST2L, the membrane form of its cognate receptor. The resulting IL-33/ST2 complex plays a role in modulating the response of cardiomyocytes and cardiac fibroblasts to biomechanical overload. This pathophysical force is currently imposed on the failing heart. In this context, activities of the IL-33/ST2 complex closely resemble those of the B-type natriuretic peptide (BNP). Both systems do their best to protect the heart from harmful cardiomyocyte hypertrophy and accumulating interstitial fibrosis. Excess levels of the soluble form of the ST2 receptor may compete with their membrane-bound counterpart for their common ligand, i.e. IL-33. Overwhelming concentrations of sST2 can thus act as a decoy receptor, representing an endogenous "off-switch" mechanism for inappropriate ST2/IL-33 signaling (31).

Outside the myocardial tissue, the ST2 receptor, irrespective of its actual form, i.e., whether membrane-bound (ST2L) or soluble (sST2):

1. is expressed on Th2, but not Th1 lymphocytes, and
2. is able to suppress both innate and adaptive immune responses (11, 12).

This particular piece of knowledge sheds new light on the interrelation between inflammatory/immune reactions and cardiovascular diseases. The following text will be focused primarily on the impact of IL-33 and Th2 immune responses on the down-modulation of atherosclerosis.

Atherosclerosis: an inflammatory and an immune disease

In an atherosclerotic vessel, both infiltrating (macrophages and T-lymphocytes) and resident cells (vascular smooth muscle cells and endothelial cells) elaborate proin-

flammatory mediators, cytokines and growth factors, in response to manifold danger signals. The most important activators of the inflammatory/immune response comprise oxidized low-density lipoproteins (ox-LDLs), heat shock proteins (HSPs) and diverse microbial products. The latter are collectively referred to as pathogen-associated molecular patterns (PAMPs) (5).

The vast majority of T-lymphocytes which infiltrate human atherosclerotic plaques belong to the CD4+ helper T cells subpopulation of TcR αβ T-cells of Th1 regulatory subset. Lymphocytes derived from naive CD4+ Th0 precursors acquire their particular phenotype after encountering antigens and costimulators in the presence of Th1-derived cytokines, mainly IL-12 and interferon (IFN)-γ (28).

To maintain a properly weighed immune balance, Th1-lymphocytes and their cytokines IFN-γ and IL-12 are off-set by **Tregs**, which are regulatory T cells that suppress activation or effector function of other T lymphocytes. The best characterized Tregs are defined by:

1. the CD4+CD25+CD127low surface phenotype
2. expression of FoxP3, a forkhead family transcription factor.

FoxP3 is a lineage-specification factor for these Tregs, imparting the suppressive capability. The FoxP3+ Tregs comprise about 5% to 10% of peripheral CD4+ T cells. They are elaborated from naive T-cell precursors both during thymic development and in the periphery, concomitantly with the generation of effector T cells. Not only are Tregs antigen-specific, but they are also in a state of permanent activation. These qualities predispose them to regulate other activated T cells:

1. by contact inhibition
2. by secretion of antiinflammatory cytokines, the most effective ones being
   - IL-10
   - transforming growth factor (TGF)-β (1).

Indeed, **IL-10** and **TGF-β** have been repeatedly demonstrated to attenuate atherosclerosis and other inflammatory/(auto)immune diseases. Moreover, transfer of Tregs from healthy donors to atherosclerotic recipients in apoE -/- mice leads to a significant attenuation of atherosclerotic plaque:

1. formation
2. extent
3. cellular infiltration

as compared to the delivery of effector CD4+CD25– cells which do not exhibit suppressive activites of Tregs (21).

A Th1/Th2 imbalance may underlie atherosclerosis

Taking these facts into account, it can be concieved that predominance of the Th2 immune response including its cytokines might slow down the onset or even decrease the extent of established atherosclerosis. An increasing body of evidence suggests that such a scenario can be expected to occur *in vivo*. In a recent study, *Libby* and coworkers presented that genetically modified mice developed either severe or mild forms of atherosclerosis according to their respective programmed biases toward either a Th1 or a Th2 immune response. Of note, plasma cholestrol levels did not differ in either group. The extent of resulting atherosclerosis could be attributed exclusively to the prevailing form of the inborn immune response (33). This study supports an older finding which showed that severe hypercholesterolemia itself skewed the cellular immune response toward the Th2 lymphocyte subset (the **Th1→Th2 shift**), presumably in a late and a vain effort to dampen atherogenesis (37). It is also in line with the concept that atherosclerosis, in its innermost nature, is a process which has evaded its inherent counter-regulatory mechanisms which are always present, but in an established form of the disease they set in too late and/or with an insufficient force (18).

On the other hand, B-lymphocytes are only rarely recovered from atherosclerotic plaques, despite the fact that many patients do exhibit systemic humoral responses to ox-LDLs or to HSPs, not to speak about elevated antibody titres against presumed atherogenic pathogens, such as:

1. *Chlamydia pneumoniae*
2. *Helicobacter pylori*
3. *Porphyromonas gingivalis*
4. cytomegalovirus (CMV)
5. virus *Herpes simplex 1* (HSV1)

to name only the most important pretendents (14).

Antibody production by B-lymphocytes is dependent on activatory signals delivered by Th2 lymphocytes and their cytokines. Consequently, B-lymphocytes are supposed to provide protection from atherosclerosis. Really, many studies confirmed atheroprotective effects of Th2-lymphocyte cytokines, such as IL-5, IL-10, IL-13 and, most recently, IL-33 (6, 8, 13).

Atheroprotective antibodies

Research teams conducted by *Hörkkö, Palinski* and *Binder* established that **IgM** antibodies against oxLDL correlated inversely with surrogate markers of cardiovascular disease (24). In murine models of atherosclerosis, IgM antibodies dominate the humoral response to oxLDL. These antibodies avidly recognize their cognate epitopes/antigens in atherosclerotic plaques. Thus, IgM antibodies can be expected to favorably modulate atherogenic processes by their ability to block the uptake of oxLDL and apoptotic cells by macrophages by way of scavenger receptors. In so doing, they are able to limit transformation of macrophages to foam cells. In particular, the prototypic natural germline IgM antibody **T15/EO6** has been shown to expand during atherogenesis in mice. T15/EO6 antibodies bind

1. phosphocholine (PC), which is present in oxidized phospholipids of oxLDL

2. the capsular polysaccharide of *Streptococcus pneumoniae* (34).

Furthermore, immunization of LDL receptor knockout (LDLR–/–) mice with pneumococcal extracts leads to a near monoclonal expansion of T15/EO6 IgM and significantly decreases atherosclerotic lesion formation (7). Immunization of atherosclerosis-prone mice or rabbits with model oxidation epitopes, such as malondialdehyde-modified LDL (MDA-LDL), has been shown to induce specific atheroprotective antibodies (25). Recently, the same authors demonstrated that immunization of LDLR–/– mice with homologous MDA-LDL led to a dominant induction of the Th2 cytokine **IL-5** and to a noncognate expansion of natural T-cell independent T15/EO6 antibodies. Most importantly with respect to the discussed topic, this expansion of T15/EO6 IgM was dependent on the presence of the Th2 cytokine IL-5, since IL-5 deficient mice did not elicit the protective response. Reconstitution of LDLR–/– mice with bone marrow from IL-5 deficient donors resulted in significantly more extensive atherosclerosis.

Thus, IL-5 unequivocally:
1. has an overall atheroprotective role *in vivo*
2. is critically involved in the production of atheroprotective natural IgM antibodies T15/EO6.

IL-5 has a central role in B-cell biology. It is important for:
1. proliferation
2. survival
3. Ig production

of murine B-1 cells, which constitutively express its receptor IL-5R. These cells are the major source of natural IgM antibodies, including T15/EO6 (20).

In contrast to mice, the role of IL-5 in human B-cell biology is less well established.

Sämpi et al. showed in a nice recent study that IL-5 plasma levels were associated with IgM antibodies to epitopes of oxLDL. This finding is suggestive of an in vivo IL-5 function in stimulating specific human IgM antibody responses. The IgM autoantibodies to oxLDL have been documented to be inversely related to measures of human atherosclerosis. By contrast, IgG to oxLDL did not show such a relationship. Thus, IL-5, supported by IL-33, may mediate a protective response by preferentially promoting anti-oxLDL IgM production. These findings demonstrated for the first time a link between plasma IL-5 levels and the titre of specific IgM antibodies to neoself epitopes in humans (30).

Finally, *Miller* and coworkers demonstrated that induction of IL-5 and atheroprotective antibodies to oxLDL depended on the cytokine IL-33.

IL-33 treatment further reduced:
1. the percentage of plaque area that stained positive for F4/80+ macrophages
2. the number of lesion-associated CD3+ T lymphocytes.

Conversely, co-administration of an anti-IL-5 antibody concomitantly with IL-33 diminished the levels of anti-oxLDL antibodies and stopped the reduction of atherosclerotic plaque size. Furthermore, apoE -/- mice treated with soluble ST2, the decoy receptor which neutralizes IL-33, developed more extensive atherosclerotic plaques that were extremely rich in inflammatory cells as compared to control IgG-treated animals. Specific antibodies to ox-LDL could not be demonstrated in these sST2-treated mice. At the same time, a significant increase in IFN-γ production by lymph node cells was revealed. To sum it up, in the established ApoE-/- mice model of atherosclerosis, IL-33 performed its atheroprotective role via a Th1→ Th2 shift of the specific cell mediated immune response (19). This is further confirmed by the capacity of IL-33 to increase the production of:
1. IL-4
2. IL-5
3. IL-13

all of them typical Th2 immune response cytokines with atheroprotective activities in parallel with IL-10 and TGF-β. Production of the last two, however, is not governed by IL-33.

As has been said above, IL-33 reduces both in serum and in lymph nodes the amounts of IFN-γ, an archetypical cytokine involved in Th1 immune responses.

Furthermore, IL-33 increases serum levels of Th2-driven specific immunoglobulin classes
1. IgA
2. IgE
3. IgG1

while it decreases levels of the Th1-driven specific immunoglobulin sub-class IgG2a (23).

Tissue/cellular origin of IL-33

Smooth muscle cells and endothelial cells are known to produce and respond to IL-1 and IL-18. As far as the third member of the IL-1 family is concerned, a RT-PCR examination of cDNA libraries in mice and men established the presence of IL-33 mRNA in
1. dermal fibroblasts
2. keratinocytes
3. coronary artery, bronchial, and pulmonary smooth muscle cells (SMCs) (3, 16).

Moreover, IL-33 can be found:
1. in ECs of patients suffering from rheumatoid arthritis with chronically inflamed synovium
2. in the intestine of Crohn's disease patients (17).

Miller and coworkers revealed by PCR and immunohistochemistry the expression of IL-33 and its receptor ST2 in human vascular cells. The authors examined the expression of mRNAs for IL-33 and for ST2 in primary cultured:
1. human umbilical vein ECs,
2. human saphenous vein ECs,
3. human saphenous vein SMCs,
4. human coronary artery SMCs.

This particular distribution is further suggestive of IL-33's role in vascular (patho)physiology (2).

Just the same as IL-1 and IL-18, IL-33 is synthesized as a 31-kDa precursor which is cleaved by caspase-1 to form

a mature 18-kDa protein. Apart from driving Th2 responses in lymphocytes, mature IL-33 was found to induce blood eosinophilia and splenomegaly. Moreover, IL-33 also induces

1. maturation and proinflammatory cytokine production in mast cells
2. degranulation and survival in eosinophils.

These findings are in line with possible adverse effects of IL-33 which involve, above all, exacerbation of bronchial asthma or even the development of anaphylactic shock (27).

As will be discussed further, IL-33 can lead to insufficient wound healing and it also seems that IL-33 might be implicated in tumor growth.

Biological effects of IL-33 discussed so far are displayed in the extracellular space by its mature 18-kDa form. However, the full-length precursor IL-33 can be active as an intracellular transcription factor with repressor properties. In endothelial cells, nuclear IL-33 is expressed mostly in resting blood vessels of healthy tissues. Activation of endothelial cells by TNF-α or IL-1β appeares to be associated with a decay of intracellular IL-33, which is:

1. rapidly down-regulated in the proinflammatory/angiogenic environment of wound healing
2. undetectable in the angiogenic/immature vessels of tumors (15).

In general, IL-33 is strongly down-regulated in endothelium undergoing tumor or wound healing angiogenesis, the more so in the presence of congested, thin-walled vessels in tissues undergoing wound healing and scar formation or in tumor tissue (36). In addition to proinflammatory cytokines, vascular endothelial growth factor (VEGF) may also be a driving force during wound healing. Prestored VEGF is released from platelets, and may act in concert with TNF-α released from tissue-resident mast cells to down-regulate vascular IL-33 (26). Conversely, inappropriate IL-33 amounts may interfere with firm scar formation in hearts which have been attacked by myocardial infarction. As it is, excess IL-33 – it remains to be proved that such quantities are ever attained – might represent a new risk factor of postinfarct heart rupture (22, 35).

To sum up this last section, IL-33 seems to be strongly expressed in nuclei of resting endothelium. However, it is rapidly down-regulated during the earliest angiogenic events in a process that involves:

1. exposure to proinflammatory cytokines TNF-α or IL-1β
2. exposure to proangiogenic VEGF
3. loss of cell-cell contacts (9).

The reduced expression of IL-33 in tumors and during wound healing may contribute to maintaining the angiogenic state of vessels which is necessary for solid tissue growth (4, 10).

Conclusion

Interleukin-33 and its receptor ST2 (IL-33α) seem to play a favorable role in the course of atherogenesis by promoting a Th1→Th2 shift of the immune response. IL-33 may also protect the heart from unfavorable biomechanical forces which lead to the muscle's distension, hypertrophy and overwhelming fibrosis. On the other hand, IL-33 shares the unwanted effects of other Th2 cytokines, namely a predisposition to allergic reactions. As a nuclear transcription factor of endothelial cells, IL-33 is abundant in resting, nonproliferating vessels. Its down-regulation seems to be necessary for angioneogenesis, whether it is profitable like during wound healing or deleterious, such as during tumor growth. However, it must be emphasized that IL-33 is a newly recognized cytokine whose precise activities, notwithstanding the growing sum of their knowledge, require further investigation.

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References

1. Ait-Oufella H, Salomon BL, Potteaux S, et al. Natural regulatory T cells control the development of atherosclerosis in mice. Nat Med 2006;12:178–180.
2. Aoki S, Hayakawa M, Ozaki H, et al. ST2 gene expression is proliferation-dependent and its ligand, IL-33, induces inflammatory reaction in endothelial cells. Mol Cell Biochem 2010;335:75–81.
3. Bartunek J, Delrue L, Van Durme F, et al. Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. J Am Coll Cardiol 2008;52:2166–2174.
4. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401–10.
5. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukocyte Biol 2007;81:1–5.
6. Binder CJ, Hartvigsen K, Chang MK, et al. IL-5 links adaptive and natural immunity specific for epitopes of oxidized LDL and protects from atherosclerosis. J Clin Invest 2004;114:427–37.
7. Binder CJ, Hörkkö S, Dewan A, et al. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL. Nat Med 2003;9:736–743.
8. Binder CJ, Shaw PX, Chang MK, et al. The role of natural antibodies in atherogenesis. J Lipid Res 2005;46:1353–1363.
9. Carriere V, Roussel L, Ortega N, et al. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. Proc Natl Acad Sci USA 2007;104:282–7.
10. Choi YS, Choi HJ, Min JK, et al. Interleukin-33 induces angiogenesis and vascular permeability through ST2/TRAF6-mediated endothelial nitric oxide production Blood 2009;114:3117–3126.
11. Fagundes CT, Amaral FA, Souza ALS, et al. ST2, an IL-1R family member, attenuates inflammation and lethality after intestinal ischemia and reperfusion. J Leukocyte Biol 2007;81:492–499.
12. Hayakawa H, Hayakawa M, Kume A, Tominaga S-i. Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation. J Biol Chem 2007;282: 26369–26380.
13. Karvonen J, Päivänsalo M, Kesäniemi YA, et al. Immunoglobulin M type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid artery atherosclerosis. Circulation 2003;108:2107–2112.
14. Krebs P, Scandella E, Bolinger B, Engeler D, Miller S, Ludewig B. Chronic immune reactivity against persisting microbial antigen in the vasculature exacerbates atherosclerotic lesion formation. Arterioscler Thromb Vasc Biol 2007;27: 2206–13.
15. Küchler AM, Pollheimer J, Balogh J, et al. Nuclear interleukin-33 is generally expressed in resting endothelium but rapidly lost upon angiogenic or proinflammatory activation. Am J Pathol 2008;173:1229–42.
16. Kurowska-Stolarska M, Kewin P, Murphy G, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. J Immunol 2008;181:4780–4790.
17. Matsuyama Y, Okazaki H, Tamemoto H, et al. Increased levels of interleukin 33 in sera and synovial fluid from patients with active rheumatoid arthritis. J Rheumatol 2010;37:18–25.

18. Merched AJ, Ko K, Gotlinger KH, Serhan CN, Chan L. Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. FASEB J 2008;22:3595–606.
19. Miller AM, Xu D, Asquith DL, et al. IL-33 reduces the development of atherosclerosis. J Exp Med 2008;205:339–46.
20. Moon BG, Takaki S, Miyake K, et al. The role of IL-5 for mature B-1 cells in homeostatic proliferation, cell survival, and Ig production. J Immunol 2004;172: 6020–6029.
21. Mor A, Planer D, Luboshits G, et al. Role of naturally occurring CD4+CD25+ regulatory T cells in experimental atherosclerosis. Arteriol Thromb Vasc Biol 2007;27:893–900.
22. Mueller T, Dieplinger B, Gegenhuber A, Poelz W, Pacher R, Haltmayer M. Increased plasma concentrations of soluble ST2 are predictive for 1-year mortality in patients with acute destabilized heart failure. Clin Chem 2008;54:752–756.
23. Murphy GEJ, Xu D, Liew FY, McInnes IB. Role of interleukin 33 in human immunopathology. Ann Rheum Dis 2010;69(Suppl.1):i43–i47.
24. Palinski W, Hörkkö S, Miller E, et al. Cloning of monoclonal autoantibodies to epitopes of oxidized lipoproteins from apolipoprotein E-deficient mice. Demonstration of epitopes of oxidized low density lipoprotein in human plasma. J Clin Invest 1996;98:800–814.
25. Palinski W, Miller E, Witztum JL. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. Proc Natl Acad Sci U S A 1995;92:821–825.
26. Pettersson A, Nagy JA, Brown LF, et al. Heterogeneity of the angiogenic response induced in different normal adult tissues by vascular permeability factor/vascular endothelial growth factor. Lab Invest 2000;80:99–115.
27. Pushparaj PN, Tay HK, H'ng SC, et al. The cytokine interleukin-33 mediates anaphylactic shock. Proc Natl Acad Sci USA 2009;106;9773–9778.
28. Read S, Powrie F. CD4+ regulatory T cells. Curr Opin Immunol 2001;13: 644–649.
29. Rehman SU, Mueller T, Januzzi JL. Characteristics of the novel interleukin family biomarker ST2 in patients with acute heart failure. J Am Coll Cardiol 2008;52:1458–65.
30. Sämpi M, Ukkola O, Päivänsalo M, Kesäniemi YA, Binder CJ, Hörkkö S. Plasma interleukin-5 levels are related to antibodies binding to oxidized low-density lipoprotein and to decreased subclinical atherosclerosis. J Am Coll Cardiol 2008;5 2:1370–8.
31. Sanada S, Hakuno D, Higgins LJ, Schreiter ER, McKenzie AN, Lee RT. IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. J Clin Invest 2007;117:1538–1549.
32. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005;23:479–90.
33. Schulte S, Sukhova GK, Libby P. Genetically programmed biases in Th1 and Th2 immune responses modulate atherogenesis. Am J Pathol 2008;172:1500–8.
34. Shaw PX, Hörkkö S, Chang MK, et al. Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity. J Clin Invest 2000;105:1731–1740.
35. Shimpo M, Morrow DA, Weinberg EO, et al. Serum levels of the interleukin-1 receptor family member ST2 predict mortality and clinical outcome in acute myocardial infarction. Circulation 2004;109:2186–90.
36. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiol Rev 2003;83:835–70.
37. Zhou X, Paulsson G, Stemme S, Hansson GK. Hypercholesterolemia is associated with a T helper (Th)1→Th2 switch of the autoimmune response in atherosclerotic apoE-knockout mice. J Clin Invest 1998;101:1717–25.

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