Increased Circulating Levels of 3-Nitrotyrosine Autoantibodies
Marker for or Maker of Cardiovascular Disease?

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3-nitrotyrosine formation is an oxidative protein modification that was first discovered in vivo in the early 1990s by Beckman and colleagues. The biological relevance of this process was extensively investigated in the subsequent years and further facilitated by the development of 3-nitrotyrosine-specific antibodies. Protein tyrosine nitration is mainly mediated by 3 biochemical processes (Figure): (1) by peroxynitrite (ONOO⁻) formation, the reaction product of nitric oxide (NO) and superoxide (O₂⁻); (2) by a (myelo)peroxidase-catalyzed nitrogen dioxide radical (NO₂) formation from hydrogen peroxide and nitrite; and (3) by a nonspecific formation of the nitrogen dioxide radical from nitric oxide in oxygenated buffers (reflecting rather artificial ex vivo conditions).

What Are the Pathophysiological Consequences of Increased Tyrosine Nitration of Proteins?
Protein tyrosine nitration represents a posttranslational modification of proteins in situations of high oxidative stress. In the setting of cardiovascular risk factors, when superoxide producing enzymes such as the NADPH oxidase are getting activated or the nitric oxide synthase is getting uncoupled, O₂⁻ will react with (and therefore metabolize) nitric oxide (NO) in an almost diffusion-controlled (this means ultrafast) reaction that even outcompetes the detoxification of O₂⁻ by superoxide dismutases. It is of clinical importance that this reaction not only forms the highly reactive intermediate peroxynitrite (ONOO⁻), one of the most potent biological oxidants, but at the same time consumes the most important vasodilator principle, NO. The superoxide/nitric oxide reaction product peroxynitrite further impairs vascular function and generates a prothrombotic state by inhibiting prostacyclin formation via tyrosine nitration (see Table I in the online-only Data Supplement in and inactivation of prostacyclin synthase, in favor of the formation of the vasoconstrictor prostaglandin endoperoxide H₂ (PGH₂). More examples for direct effects of tyrosine nitration on enzymatic function and properties have been reported recently: Fibrinogen nitration has been demonstrated to increase the extent of fibrin clot formation, and oxidatively modified apolipoprotein A-I was shown to cause a dysfunctional form of high-density lipoprotein with a diminished capacity to generate NO, with reduced antioxidant capacity and 3-nitrotyrosine modified apolipoprotein A-I leads to diminished ATP binding cassette transporter A-1 cholesterol efflux capacity from macrophages, and reduced lecithin cholesterol efflux acyl transferase activity, all of which will create a prothrombotic and proatherosclerotic milieu.

For many years it has been known that nitrated proteins are able to stimulate the formation of specific antibodies (this is the procedure how specific diagnostic antibodies against 3-nitrotyrosine-positive proteins are generated) and that protein nitration as a posttranslational modification, will further trigger immune reactions, which has been demonstrated for certain rheumatic diseases such as rheumatoid arthritis or systemic lupus erythematosides. The question whether there is an association between coronary artery disease (CAD), a disease that is also linked with chronic inflammation and activation of the immune system, and increased levels of immunoglobulins reactive against nitrotyrosine-positive proteins has never been addressed so far.

With the present studies, Thomson et al demonstrate for the first time that nitrated proteins exist in human atherosclerotic plaques of the carotid arteries, which could serve as antigenic neoepitopes and potentially trigger the activation of the immune system. The authors also demonstrate that levels of circulating immunoglobulins against 3-nitrotyrosine epitopes were strikingly (10-fold) higher in patients with CAD as compared with patients without CAD and that these levels were strongly associated with angiographic evidence of significant CAD. The specificity of their methodology was established by the following:

- The outcompeting of the 3-nitrotyrosine binding activity of the plasma by free 3-nitrotyrosine as well as by 3-nitrotyrosine-modified proteins and peptides.
By the demonstration that a nitrated peptide reflecting an endogenous nitrated tyrosine residue identified in apolipoprotein A-I recovered from human atherosclerotic plaque, but not the native tyrosine-containing peptide, was able to outcompete the binding of the 3-nitrotyrosine-coupled HRP to immunoglobulins. By the demonstration that the circulating immunoglobulins were able to recognize nitrated fibrinogen and other nitrated proteins but not the native counterparts.

The mechanism for the production of immunoglobulins with selective and specific epitope recognition for 3-nitrotyrosine has been identified previously to be a consequence of termination of self-tolerance and escape of negative selection. The results of the present investigations just indicate that increased circulating immunoglobulins targeting protein-bound 3-nitrotyrosine are increased in patients with significant CAD.

Conclusions and Perspectives

Although these findings are pretty exciting, the study delivers no answer to the question regarding whether this antibody formation response can be considered as a marker or a maker of CAD, nor whether the increased antibody response may even lead to a stabilization or a destabilization of the atherosclerotic plaque and therefore to more or even fewer cardiovascular events.

It also brings up the chicken and egg question: do the antibody levels rise before overt atherosclerosis development, or are they a consequence of already established atherosclerosis? It would be also important to see whether the levels are increased in patients with essential hypertension without overt cardiovascular disease and how these levels may respond in patients with CAD and hypertension to antiatherosclerotic and antihypertensive therapy, such as statins, angiotensin-converting enzyme–inhibitors, or angiotensin II receptor blockers. Nevertheless, the presented results demonstrating increased levels of anti–3-nitrotyrosine–positive proteins may be used as a new biomarker for the detection of the activity of the atherosclerotic process and may also represent a new target for cardiovascular disease treatment (eg, by using antioxidants as suppressors of protein tyrosine nitration). Tyr indicates tyrosine; 3NT, 3-nitrotyrosine.

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Disclosures

None.

References


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