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Is Osteopontin a Good Marker for Graves' Disease?

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SUMMARY

Background

CD40 is a receptor expressed on various antigen-presenting cells, such as the B cell. If a B cell presents an antigen recognized by an activated CD4+ T cell, the ligand for CD40 (CD40L) that becomes expressed on the T-cell stimulates the B cell to multiply and form plasma cells that produce copious amounts of antibody against the target antigen (for example, the TSH receptor). CD40L can be expressed on the surface of many cell types and also can be partly cleaved by proteases and released into the circulation. Both the surface-bound and the soluble forms of CD40 ligand have biologic activity. CD40, a member of the tumor necrosis factor (TNF)–receptor family, also has been detected on thyrocytes, and a single-nucleotide polymorphism in the CD40 gene may increase the risk of Graves' disease. Thus, understanding how CD40/CD40L interactions are regulated may help in understanding the pathophysiology of Graves' disease.

Osteopontin (not to be confused with osteocalcin) is a cytokine associated with many autoimmune and chronic inflammatory diseases. It can bind to cells via several surface receptors, including beta integrins on T cells, and it promotes the migration of lymphocytes and their production of cytokines. The authors of the current article previously showed that the level of osteopontin was elevated in Graves' disease and was closely associated with Graves' disease activity, prompting them to suggest that it could be a biomarker for active Graves' disease (1). In the present article, they examined the effects of plasma from patients with Graves' disease—and the effects of synthetic osteopontin—on the expression of CD40L in plasma and on CD4+ T cells, and on the production of immunoglobulins by peripheral-blood mononuclear cells (PBMCs).

Methods

Blood from 40 patients with active Graves' disease, 21 patients with Graves' disease in remission after 6 months of MMI treatment, and 27 healthy subjects was drawn into EDTA-containing tubes on ice and immediately centrifuged to obtain platelet-free plasma. The level of CD40L expressed on the surface of CD4+ T cells (prepared from PBMCs) was measured using flow cytometry. The levels of soluble CD40L and osteopontin were measured by ELISA and were correlated with laboratory measures of Graves' activity. PMBCs were incubated for a day with normal plasma, with plasma from patients with untreated Graves' disease (final dilutions, 1:4), or with recombinant human osteopontin, and also with anti-osteopontin or control antibody. The surface expression of CD40L on CD4+ T cells was then assessed by flow cytometry on the PBMCs. Other PBMCs were incubated with the same agents for 12 hours, and the level of CD40L mRNA was measured by RT-PCR on CD4+ T cells obtained by flow cytometry. Still other PBMCs were incubated with osteopontin with or without anti-CD40L monoclonal antibody, and after 10 days' culture, the incubation media were analyzed for IgG and IgM levels.

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Results

The levels of both soluble and membrane-bound CD40L were 3 times as high in patients with active Graves' disease as in patients in remission or in normal controls. The plasma level of osteopontin correlated closely with the level of soluble CD40L. Both the plasma level of CD40L and that of osteopontin correlated positively with free T_4, free T_3, anti-TPO, anti-Tg, and anti-TSH-receptor levels and correlated negatively with TSH levels. For membrane-bound CD40L, the correlations were similar, except that those with anti-TPO and anti-Tg were not significant and the correlation with osteopontin was not as tight. The mean basal expression of membrane-bound CD40L on CD4+ T cells from patients with active Graves' disease was about twice that in controls (5 in each group). Treating these CD4+ T cells with osteopontin or with plasma from patients with active Graves' increased the surface expression of CD40L, whereas adding a monoclonal antibody against osteopontin blocked the responses. The levels of mRNA encoding CD40L responded similarly to these combinations of agents. The secretion of IgG was about 50% greater in the medium from unstimulated Graves' PBMCs as compared with control cells. Stimulation with osteopontin increased IgG levels by about 70% in the medium from Graves' PBMCs and about 60% in controls. Baseline IgM levels were about twice as high in Graves' cell media than in controls. Osteopontin stimulated IgM levels 7-fold in medium from Graves' PBMCs and 2.5-fold in controls. Adding anti-CD40L antibody blocked the IgG and IgM responses to osteopontin.

Conclusions

Plasma osteopontin levels correlate positively with anti-TSH-receptor antibody levels and negatively with TSH levels in patients with Graves' disease. Adding osteopontin or plasma from patients with active Graves' disease to CD4+ T cells increases both the membrane expression and the mRNA for CD40L, whereas adding anti-osteopontin monoclonal blocks these responses. Osteopontin induces a rise in CD40L that in turn increases the production of immunoglobulins from PBMCs.

ANALYSIS AND COMMENTARY

The plasma level of osteopontin may not be a very selective biomarker for Graves' disease, because in addition to its connection with bone turnover, it can also be regulated by hypoxia, transforming growth factor β, TNF-α, interleukin-1β, angiotensin II, nitric oxide, and hyperglycemia. Furthermore, a recent study found serum osteopontin levels to be increased in hyperthyroidism but to be decreased in hypothyroidism (2), and experimental studies indicate that osteopontin levels rise and fall together with the thyroid hormone level, so the current findings probably reflect the altered thyroid hormone levels rather than Graves' disease per se. In view of the fact that multiple isoforms of osteopontin (and CD40L) exist, their roles probably deserve further exploration. Nonetheless, the increased level of osteopontin could well be connected with some of the effects of Graves' disease on the skeletal, adipose, and cardiovascular systems.

The same research group also recently reported that the plasma level of the cytokine CCL20 is increased in Graves' disease (3). The CCL20 level correlated with plasma osteopontin levels, and recombinant osteopontin increased expression of CCL20 mRNA in CD4+ T cells. Adding plasma from patients with untreated Graves' disease increased the CCL20 expression 3-fold as compared with normal plasma, and this response was blocked by antibodies to osteopontin as well as to β3 integrin (a receptor for osteopontin) and also by inhibitors of the nuclear factor κB and mitogen-activated protein kinase pathways. The authors again suggest that CCL20 could be a biomarker for Graves' disease. In passing, one might note that both osteopontin and CCL20 have been reported to be overexpressed in RET/PTC papillary thyroid cancer, suggesting a possible connection with the recently discussed report of increased aggressiveness of cancers associated with Graves' disease (4).

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