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Elevated Concentrations of Interleukin-1β and Interleukin-1 Receptor Antagonist in Plasma of Women with Silicone Breast Implants

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Plasma from 27 women with silicone breast implants (SBIs) and 50 age-matched control women without SBIs were examined by enzyme immunoassay for the presence of interleukin-1β (IL-1β) and its naturally occurring receptor antagonist, IL-1ra. The results show that 74% (20 of 27) of women with SBIs had elevated concentrations of IL-1ra, whereas only 2% (1 of 50) of controls without SBIs had elevated concentrations of IL-1ra. In contrast to the IL-1ra results, the frequency of elevated IL-1β concentrations among women with SBIs was only 40% (11 of 27), but this was significantly higher than the 0% (0 of 50) in control women without SBIs. These findings suggest that there is a chronic ongoing inflammatory process in some women with SBIs, the implications of which are discussed in the context of silicone as an antigenic stimulant of the immune system.

Many human diseases are characterized by excessive, persistent, or inappropriate immune responses. Among the cells involved in the responses are monocytes, neutrophils, basophils, eosinophils, and lymphocytes, which originate from the blood, and the endothelial cells, mast cells, tissue fibroblasts, and resident macrophages, which originate locally. Many of these cells produce proinflammatory cytokines which can contribute to the pathogenesis of inflammatory autoimmune diseases such as rheumatoid arthritis (8).

"Silicone" refers to a group of compounds including fluids, gels, rubbers, sponges, foams, and resins (3). Silicones are never found in nature but are derived from quartz, also known as silica (9). The fluid and gel are used as a polymer (dimethylpolysiloxane) in breast implants (2). The inflammatory effects of silicon (silicone) exposure are well documented in animals (13, 16). In humans, rheumatic autoimmune syndromes are reported in some women with silicone breast implants (SBIs) (5, 19). While the adjuvanticity of silicones is well known, the specific immune responses elicited by silicone compounds (e.g., silica, silicates, and silicone) were only recently described (14, 18). Basic questions about how silicone compounds interact specifically with the immune system are now being asked. To this end, plasma from 27 women with SBIs and 50 women without SBIs were assayed for the proinflammatory cytokine interleukin-1β (IL-1β) and its receptor antagonist, IL-1ra. These analytes were chosen because IL-1 is known to be inducible by silica (7) and IL-1ra neutralizes the effects of IL-1.

MATERIALS AND METHODS

Reagents. Commercial solid-phase enzyme immunoassay (EIA) kits for IL-1β and IL-1ra were purchased from R&D Systems, Minneapolis, Minn.

Plasma donors. Plasma was obtained from peripheral blood in heparinized tubes by venipuncture from 50 healthy control women (ages, 22 to 45 years) without SBIs and 27 age-matched women with SBIs. The samples from women with SBIs were remnant samples from samples sent to Specialty Laboratories, Inc., for routine clinical testing. Plasma samples from both women with SBIs and control women were kept frozen at −20°C until the time of assay. Because the samples from women with SBIs were routine clinical samples, no clinical data on them were available except the fact that they all had breast implants.

Determination of IL-1ra and IL-1β by EIA. (i) IL-1ra. The R&D Systems Quantikine EIA kit was used to measure IL-1ra levels in plasma. This kit uses the quantitative sandwich EIA technique. Briefly, a monoclonal antibody specific for IL-1ra was coated onto microtiter plates. A serially diluted recombinant human IL-1ra standard provided by the manufacturer (3,000 to 93.8 pg/ml) and undiluted samples in 200 μl were added to the wells. After washing away unbound proteins, an enzyme-linked polyclonal antibody specific for IL-1ra was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells to develop the color in proportion to the amount of IL-1ra bound in the initial step. The optical density (OD) of each well was determined by an enzyme-linked immunosorbent assay reader set to 450 nm. A standard curve was prepared by plotting the OD versus the concentration of IL-1ra in the standard wells. By comparing the OD of the samples with the standard curve, the concentration of the IL-1ra in the unknown samples was determined.

(ii) IL-1β. The R&D Systems Quantikine EIA kit was used to measure IL-1β levels in plasma. The assay procedure used was essentially the same as that described above for IL-1ra, except that a monoclonal antibody specific for IL-1β was coated onto the microtiter plate. The conjugate used to sandwich the immobilized IL-1β was an enzyme-linked polyclonal antibody specific for IL-1β. A standard curve was prepared by plotting the OD versus the concentration of IL-1β in the standard wells. By comparing the OD of the samples with this standard curve, the concentration of the IL-1β in the unknown samples was determined.

Establishment of inter- and intra-assay coefficients of variation (CVs) for IL-1ra and IL-1β EIAs. (i) Interassay precision (precision between assays). Three samples with known concentrations were assayed 20 times to assess interassay precision.

(ii) Intra-assay precision (precision within an assay). Three samples with known concentrations were assayed in replicates of 20 to assess intra-assay precision.

Determination of normal reference range. On the basis of the mean EIA values (in picograms per milliliter plus 2 standard deviations) for 50 healthy control women without SBIs, a cutoff of 900 pg/ml was established for IL-1ra and a cutoff of 5 pg/mL was established for IL-1β.

Statistical analysis. Data were analyzed by Student's *t* test to determine the level of significance. Regression analysis was used to determine whether there was a correlation between IL-1β and IL-1ra levels.

RESULTS

Assays for IL-1β and IL-1ra. The minimum detectable concentration of IL-1β was 0.3 pg/ml, whereas that of IL-1ra was 22.0 pg/ml. The inter- and intra-assay CVs for IL-1β were less than 5%. The interassay CV for IL-1ra was 5.4%, while the

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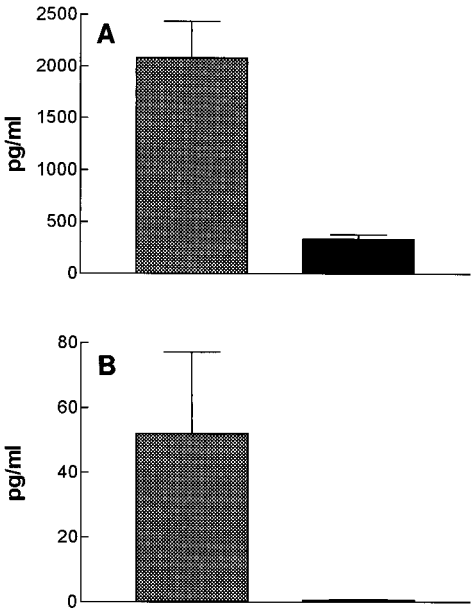

FIG. 1. Concentrations of IL-1ra (A) and IL-1β (B) in the plasma of women with SBIs (▨) and in the plasma of women without SBIs (■). The results are presented as means ± standard errors.

intra-assay CV was 4.9%. The mean concentration of IL-1ra in 27 women with SBIs was 2,070 ± 355 pg/ml, compared with a mean concentration of 340 ± 40 pg/ml ($P < 0.0001$) in the plasma of 50 women without SBIs (Fig. 1A). Of the 27 women with SBIs tested, 20 (74%) had IL-1ra concentrations greater than 900 pg/ml, whereas only 1 of 50 (2%) women without SBIs had a similarly elevated concentration. The mean concentration of IL-1β in plasma was 52 ± 25 pg/ml in 27 women with SBIs, whereas the mean concentration was 0.50 ± 0.20 pg/ml in the plasma of 50 women without SBIs ($P < 0.05$) (Fig. 1B). There was more than a 100-fold difference between the IL-1β concentrations in the plasma of women with and women without SBIs; the frequencies of elevated plasma IL-1β concentrations (>5 pg/ml) were 40 and 0% in women with and women without SBIs, respectively. There was no correlation between IL-1β and IL-1ra levels in women with SBIs ($r^2 = 0.34$).

DISCUSSION

The finding of autoantibodies in populations of women with SBIs has given rise to appellations such as siliconosis, human adjuvant disease, and silicone-related autoimmune disorders (20), which suggest that immunological disturbances could be responsible for the signs and symptoms of the SBI syndrome. Recently, we reported that silicone-specific T-cell activation occurs in a subpopulation of women with SBIs (14). These findings were confirmed by other workers (18), who showed that silica could induce specific T-cell stimulation in vitro in the blood of women with SBIs (18). Thus, a link was established between silicone and immune responses in women with SBIs but not in controls. The mechanisms relating these findings and autoantibody formation to clinical symptoms remain to be elucidated. Recently, some investigators emphasized that workers occupationally exposed to silica have a high probability of developing a spectrum of clinical and serological autoimmune manifestations, including scleroderma (17). Furthermore, silica and silicone compounds are known to induce monocyte/macrophage activation to produce IL-1 (12).

In the present report, high concentrations of both IL-1β and its receptor antagonist, IL-1ra, were detected in the plasma of some women with SBIs, thereby providing direct evidence for the activation of the monocyte/macrophage/T-cell axis. It is our thesis that in women with SBIs, there is a slow and persistent release of silicone compounds (silicone, silica, or silicates) from the implant site into the lymphatics, lymph nodes, and the immune system, with the resultant production of interleukins and other cytokines both locally and systemically. Overproduction or a deficiency of certain cytokines is thought to contribute to the pathogenesis of autoimmune diseases, either directly, by causing tissue destruction, or indirectly, through the activation of autoreactive and inflammatory cells (1, 4, 15). Recently, it was reported that high concentrations of IL-1β are produced by synovial fluid neutrophils from patients with rheumatoid arthritis (4). Kumagai and associates (10) first suggested the association of scleroderma and liquid silicone injection augmentation.

The overproduction of IL-1ra (as found in the majority of women in the present study) could actually have a beneficial effect by downregulating the adverse effects of IL-1β, such as fever, inflammatory reactions present in the joints of patients with rheumatoid arthritis, and T- and B-cell activation (7). In support of this notion is the finding that deficient IL-1ra production can contribute to persistent joint synovitis by a failure to modulate the effects of IL-1β (6). It is noteworthy that a recent open study has shown a beneficial effect of IL-1ra on rheumatoid arthritis (11). Future studies must focus on the possible relationship between high concentrations of IL-1β and/or IL-1ra and the severity of clinical symptoms or signs of disease in subpopulations of women with SBIs.

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