Eosinophilia and Cellular Cytokine Responsiveness in Hemodialysis Patients1,2

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ABSTRACT

Eosinophilia in hemodialysis (HD) patients has been associated with allergy to dialyzers and exaggerated activation of complement during HD. Its etiology, however, remains unknown. Complement activation can lead to cytokine production, and interleukin-2 (IL-2) administration has been shown to cause eosinophilia. Because abnormalities in cellular cytokine production in renal patients were previously demonstrated, the relationship between dialysis-associated eosinophilia and IL production in this HD population was studied. Twelve patients on chronic HD therapy with normal eosinophil counts (mean, 0.23 ± 0.03 cells/nL) were compared with nine patients with eosinophilia (mean, 0.85 ± 0.17 cells/nL). Measurements of cellular IL-1 and IL-2 production were performed before (pre) and after (post) HD with cuprammonium dialyzers. In patients with eosinophilia, stimulated cellular IL-1 production increased by 117 ± 40% (P < 0.01) when post-HD measurements were compared with pre-HD values and IL-2 production increased by 127 ± 65% (P < 0.05). In contrast, there was no difference in stimulated cellular cytokine production when values before and after HD were compared in patients without eosinophilia. Individual responses were reproducible during subsequent dialysis. It was concluded that cellular cytokine production in response to HD is not uniform. Eosinophilia is a clinically useful marker of exaggerated HD-associated cytokine production. Cytokine production depends on individual responsiveness and is probably related to atopy. Cytokines may play an important role in the pathogenesis of HD-associated eosinophilia and hypersensitivity.

Key Words: Interleukin-1, Interleukin-2, cuprammonium

1 Hemodialysis (HD)-induced leukopenia and eosinophilia have long been recognized, but their clinical significance has been uncertain (1,2). Complement activation has been associated with many adverse effects, such as leukopenia, hypotension, hypoxemia, pruritus, hypersensitivity reactions, and edema (3-6). The etiology and significance of HD-associated eosinophilia, however, remains unclear.

Recent biocompatibility studies have focused on the production of cytokines, peptide regulators released from a variety of cells, including activated leukocytes. The most well-studied cytokines are interleukin-1 (IL-1) and interleukin-2 (IL-2). The normal kidney has important regulatory and excretory functions for many hormones and small-molecular-weight proteins such as cytokines (polypeptides sized between 10,000 and 25,000 d) (7). Ninety percent of administered IL-1 was excreted by the kidney in a murine model (8). The half-life of IL-2 greatly increases after ligation of the renal arteries. IL-2 may be metabolized by the renal tubule (9,10).

Cytokines are important mediators of the immune response. Their activation has been proposed as an explanation for both acute and chronic complications in patients with ESRD treated with HD (11-13). Circulating levels and cellular production of both cytokines are generally, though not consistently, reported to be increased in uremia and ESRD (14-20). The role of HD in the abnormal control of cytokine regulation is not clear, because patients with chronic renal failure, before ESRD therapy is initiated, have evidence of abnormal stimulated cellular IL-1 and IL-2 production (18).

Eosinophilia has been reported after the administration of recombinant IL-2 (21-24) and is also commonly found in HD patients (25-28). Because HD induces cytokine production including IL-2 (15,18), we studied the relationship between cellular cytokine production and dialysis-associated eosinophilia in our patients.
METHODS

Patients

Twenty one patients with ESRD ranging in age from 35 to 71 yr (mean, 56.9 ± 2.6 yr) were studied. Fifteen were male, and 6 were female; 20 were black, and 1 was white. The underlying disease was chronic glomerulonephritis in nine, hypertension in seven, diabetes mellitus in three, and chronic interstitial nephritis in two. All patients were treated with thrice-weekly chronic maintenance HD for 1 to 166 months (mean, 51.4 ± 10.3 months). All were hemodialyzed with cuprammonium dialyzers (5N or 6N; Gambro, Lund, Sweden) with an acetate bath. Five milliliters of whole blood was drawn before (pre) and after (post) a 3- to 4-h HD treatment at the time of routine monthly laboratory testing. All patients gave their informed consent. The study was approved by the George Washington University Medical Center Committee on Human Research.

Eosinophil Counts

Eosinophil counts were measured with an automated cell cytometer (H-1 System; Technicon, Tarrytown, NY). The eosinophil counts are expressed in cells per nanoliter. Eosinophil counts were measured concomitantly with the cytokine measurements at the time of monthly routine laboratory evaluation in 17 of 21 patients. The presence of eosinophilia was defined as 0.38 cells/nL or more. A normal eosinophil count was defined as 0.37 cells/nL or fewer. This cut-off has been found useful previously in patients with ESRD (29). Patients with an underlying disease associated with eosinophilia (such as drug reactions or parasitic diseases) were excluded.

In order to assure that the study group with eosinophilia and the control group without eosinophilia were different, six monthly eosinophil counts preceding the measurement of IL were reviewed. Patients with both persistent and intermittent eosinophilia were included in the eosinophilia group, if averaged eosinophil counts from the patients' three highest of the preceding six monthly values were more than 0.38 cells/nL.

Cytokine Assays

As previously described (18), cellular IL-1 and IL-2 production was measured by bioassay by the method of Clemens, Morris, and Gearing (30) with CRL 1445 IL-1-dependent fibroblasts (31) and CTLL IL-2-dependent lymphocytes (32), respectively, as target cells. Briefly, whole blood was collected in citrated glass tubes, mononuclear cells were separated by density centrifugation on Ficoll-Hypaque (Pharmacia, Piscataway, NJ), and their viability was checked by trypan blue exclusion (33). The cell suspension was adjusted to 5 × 10⁶ cells/mL in medium supplemented with 10% autologous serum before the total cell volume was divided into two batches. The cells were incubated at 37°C, and the media were replenished before one batch was pulsed with 10 μg/mL of lipo polysaccharide for the measurement of IL-1 and the other was pulsed with 50 μg/mL of phytohemagglutinin for the measurement of IL-2 for a further 24 h at 37°C. After this second incubation, the cells were centrifuged and the supernatants were used in the stimulation assay. One hundred microliters of each supernatant was serially diluted and incubated with either the CRL or CTLL target cell cultures for 48 h at 37°C. The target cells were centrifuged, replenished with fresh media, and pulsed with tritiated thymidine for 24 h at 37°C. The target cells were harvested, and the amount of incorporated radioactivity was measured in a Beckman LS1308 scintillation counter (Beckman Instruments, Palo Alto, CA). Data are expressed as tritium incorporation in counts per minute (cpm) per culture.

Intracellular IL-2 levels were also measured by an antigen capture ELISA (34) with an anti-human IL-2 antibody solid-phase monoclonal antibody (35) (Advanced Biotechnologies, Columbia, MD) as both the solid-phase capture reagent and the reporter agent. The ELISA was also used to measure intracellular IL-2 levels after sonic disruption and ultracentrifugation clarification of the supernatant. The samples were compared with a standard curve, constructed by incubating the target cells with known amounts of human IL-2 (Advanced Biotechnologies). Data are expressed as tritium incorporation in counts per minute and nanograms of IL-2 per milliliter as measured by ELISA.

Data were analyzed by t test comparing differences between pre-HD and post-HD treatment values (paired t test) and comparing patients with eosinophilia and those without (unpaired t test). Cytokine levels and eosinophil counts determined at the same HD treatment were used in regression analyses. Correlations were assessed by simple regression and were analyzed by the coefficient of variance. For calculations when the magnitude of cytokine production was more important than the absolute values, the differences (deltas Δ) between post-HD and pre-HD values were assessed. Alpha <0.05 was chosen as the level of significance. Data are expressed as mean ± standard error.

RESULTS

Eosinophilia

The mean eosinophil count in all study subjects was 0.49 ± 0.10 cells/nL (range, 0.07 to 1.71 cells/nL). Twelve patients had normal eosinophil counts with a mean of 0.23 ± 0.03 cells/nL (range, 0.07 to 0.37 cells/nL). Their age was 55.4 ± 3.3 yr, and they
had been treated with HD for 40.8 ± 8.8 months. Nine patients had eosinophilia, with a mean eosinophil count of 0.85 ± 0.17 cells/nL (range, 0.38 to 1.71 cells/nL). These patients were 58.9 ± 4.4 yr of age and had been treated with HD for 65.4 ± 20.9 months. There was no significant difference between patients with or without eosinophilia in terms of race, age, sex, underlying disease, age, or time on HD.

IL-1

Stimulated cellular IL-1 production in patients with and without eosinophilia, before and after HD, is shown in Figure 1. Patients without eosinophilia before HD (pre-HD) had mean lipopolysaccharide-stimulated IL-1 levels of 10,716 ± 1,031 cpm (range, 7,006 to 20,511 cpm). Patients with eosinophilia had pre-HD IL-1 values of 13,371 ± 2,849 cpm (range, 5,901 to 32,196 cpm). There was no difference between pre-IL-1 levels in the two groups. After HD (post-HD), mean stimulated cellular IL-1 production in patients without eosinophilia was 11,319 ± 1,212 cpm (range, 5,799 to 21,333 cpm). The 9.2 ± 9.9% increase compared with mean pre-HD values was not significant. In contrast, patients with eosinophilia had mean post-HD IL-1 values of 27,123 ± 5,430 cpm (range, 8,063 to 47,035 cpm)—an increase of 116.7 ± 39.7% compared with pre-HD values (P < 0.01). Patients with eosinophilia had a greater difference between pre-HD and post-HD values of IL-1 (ΔIL-1) (13,753 ± 3,912 cpm) than patients without eosinophilia (602 ± 1,071 cpm) (P < 0.01).

IL-2

Stimulated cellular IL-2 production in patients with and without eosinophilia, before and after HD, is shown in Figure 2. Patients without eosinophilia had mean phytohemagglutinin-stimulated IL-2 measurements of 10,053 ± 389 cpm before dialysis (range, 8,000 to 12,631 cpm). Patients with eosinophilia had pre-HD IL-2 values of 12,278 ± 1,962 cpm (range, 5,886 to 26,190 cpm). There was no difference in mean pre-IL-2 values between the two groups. Patients without eosinophilia had a mean post-HD IL-2 value of 10,796 ± 1,063 cpm (range, 5,667 to 18,066 cpm). The 7.2 ± 8.8% increase compared with pre-HD values was not significant. Patients with eosinophilia had a mean post-HD IL-2 value of 24,735 ± 5,840 cpm (range, 6,211 to 53,855 cpm)—an increase of 127.2 ± 65.2% compared with pre-HD values (P < 0.05). Patients with eosinophilia had a greater difference between mean pre-HD and post-HD values of IL-2 (ΔIL-2) (12,457 ± 5,218 cpm) than patients without eosinophilia (743 ± 957 cpm) (P < 0.02).

IL-2 by ELISA

IL-2 bioassay measurements were confirmed by ELISA. The correlation between the two methods was
0.98 (P < 0.001). Patients without eosinophilia had mean pre-HD IL-2 values of 32.5 ± 2.2 ng/mL (range, 23 to 45 ng/mL). Patients with eosinophilia had pre-HD IL-2 values of 39.0 ± 6.4 (range, 14 to 75 ng/mL). There was no difference between mean pre-HD values in the two groups. After HD, patients without eosinophilia had mean post-HD IL-2 measurements of 37.3 ± 5.3 ng/mL (range, 14 to 74 ng/mL). The increase of 12.5 ± 10.6% compared with mean pre-HD values was not significant. Patients with eosinophilia had a mean post-HD IL-2 value of 83.6 ± 19.7 ng/mL (range, 18 to 164 ng/mL)—an increase of 178.0 ± 104.9% compared with pre-HD values (P < 0.05). Patients with eosinophilia had significantly greater post-HD IL-2 values (P < 0.02) and differences between mean pre-HD and post-HD (ΔIL-2) values of stimulated cellular IL-2 production by ELISA (44.6 ± 19.0) than did patients without eosinophilia (4.8 ± 4.1 ng/mL) (P < 0.05).

Correlation Between Cytokine Measurements and Eosinophil Counts

There was no correlation between individual paired absolute eosinophil counts and cytokine values before HD in the 17 patients who had simultaneous determinations. Post-HD IL-1 values, however, correlated with eosinophil counts (r = 0.60; P < 0.02). There was no significant correlation of IL-2 and eosinophil counts after HD (r = 0.37; P = 0.15). Eosinophil counts and IL-1 values correlated with the difference between post-HD and pre-HD levels (Δ) (r = 0.74; P < 0.001) (Figure 3), but the correlation between eosinophil counts and ΔIL-2 values did not reach significance (r = 0.44; P = 0.08) (Figure 4).

Correlation Between IL-1 and IL-2 Measurements

Because IL-1 production by macrophages may affect IL-1 production by lymphocytes, the relationship between the two cytokines was also analyzed by correlating paired stimulated cellular IL-1 and IL-2 production levels. Stimulated cellular IL-1 and IL-2 production correlated before (r = 0.70; P < 0.001) and after HD (r = 0.91; P < 0.001). The difference between cellular IL-1 and IL-2 production was correlated as well (r = 0.89; P < 0.001). This correlation between ΔIL-1 and ΔIL-2 was significant when patients without eosinophilia (r = 0.77; P < 0.01) or with eosinophilia (r = 0.86; P < 0.01) were analyzed separately. The slopes and the intercepts of the regression lines were not different when patients with and without eosinophilia were compared (Figure 5).
Reproducibility of Patients' Individual Cytokine Responsiveness

In order to test whether an individual's cytokine response to HD was reproducible during subsequent treatments, nine patients were evaluated a second time a month later. There was no difference in mean stimulated cellular cytokine measurements between the original and the repeat evaluations by paired t test (data not shown). Patients' individual levels of cellular cytokine production were reproducible, as reflected by the correlation of the absolute change (Δ) comparing the initial and the repeat measurements (r = 0.87; P < 0.01 for IL-1; r = 0.94; P < 0.001 for IL-2).

Six of these nine patients had eosinophilia. There was no difference between the first and the repeat evaluations when mean pre-HD and post-HD IL-1 and IL-2 levels were compared by paired t test. The absolute difference in cellular IL-1 production (ΔIL-1) was 15,399 ± 5,222 cpm (104 ± 49% increase) during the first evaluation and 13,730 ± 4,507 (93 ± 26% increase) during the repeat evaluation. There was a linear correlation of the original and the repeat ΔIL-1 evaluations (r = 0.83; P < 0.05). The absolute difference in cellular IL-2 production (ΔIL-2) was 12,754 ± 6,364 (92 ± 47% increase) during the first evaluation and 16,936 ± 7,341 (120 ± 43% increase) during the repeat evaluation. As with IL-1, the correlation of ΔIL-2 between the two evaluations was significant (r = 0.93; P < 0.01). There was no change in cellular cytokine production between the initial and repeat evaluations in three patients without eosinophilia.

DISCUSSION

Stimulated cellular cytokine production in response to HD is not uniform. This study demonstrates that dialysis-associated eosinophilia may be a marker of individuals with exaggerated cytokine responsiveness. Even though our sample population was small, we generally found that the higher the eosinophil count was, the more active was the individual's cytokine response. The patients with eosinophilia, however, did not have a uniform response. One possible explanation may be that eosinophils have both regulatory and effector roles and have been reported to down-regulate immunoglobulin (lg) E-mediated allergic responses (36–38). Because cytokines are involved in the production of lg, including lgE, it is conceivable that eosinophils may also down-regulate the cytokine response (39–42). An alternate explanation is that patients may produce cytokines in response to exposure to an allergen during dialysis, as has been reported to occur at the site of dermal injection of allergens in atopic dermatitis (43,44). The eosinophilia in patients with minimal HD-associated cytokine production might have been related to an environmental allergen, thus resulting in less cytokine production than in patients who were exposed to an allergen associated with HD.

There was a strong linear correlation between the degree of stimulated cellular IL-1 and IL-2 production both for patients with and without eosinophilia. Patients with a hypersensitive immune response may produce not only one mediator of inflammation, but a whole cascade of mediators. Patients with a history of atopy, with allergic symptoms during dialysis or with laboratory evidence of allergy such as eosinophilia, elevation in circulating IgE levels, or positive Radio-Allergo-Sorbent Tests for ethylene oxide had exaggerated complement activation during HD with cuprammonium membranes (5,29,45). Patients without evidence of atopy had minor complement activation. Our study demonstrates that cytokine production during HD is not uniform in all patients. As with complement, patients can be divided into high- and low-cytokine responders (45). HD-induced complement activation has been shown to stimulate cytokine production (46,47). The presence of eosinophilia was a marker for high-response patients. Similar to complement activation, cytokine-mediated activation of the immune system in response to HD may depend on the presence or absence of atopy in individual patients.

The determinants of dialysis-induced cytokine production are currently unknown. It may depend on the membrane material used (48–52), backfiltration of endotoxin or its fragments (53–55), or the effect of the dialysate (14,56). Others found no clear evidence of in vivo HD-induced cytokine production (16,57,58). Both HD and continuous ambulatory peritoneal dialysis result in increased capacity for cellular cytokine production (18,59); however, patients treated with peritoneal dialysis are exposed only to sterile dialysate. Because neither dialysis membranes nor endotoxin is associated with peritoneal dialysis treatment, the role of the dialysate in stimulating cytokine activation is strengthened, although Limulus lysate assay-negative endotoxin fragments cannot be excluded as a cause for cytokine activation in peritoneal dialysis. The presence or absence of allergy and/or atopy may, however, be more important than the type of stimulus to which a patient is exposed.

The growing interest in the mechanisms of immune regulation and their role in human diseases has focused on cytokine activity, the mechanisms that regulate their production, and the consequences of excessive cytokine production. Repeated activation of the immune system in HD patients provides a unique opportunity to study the acute and chronic effects of cytokine activation in vivo. HD-associated eosinophilia has long been recognized, but its etiology
IL-5 is now known to be the primary growth factor for eosinophils (38,60,61). Eosinophils express IL-2 receptors (62), but the ability of human recombinant IL-2 to induce eosinophilia seems to be mediated mainly by IL-5 because it can be inhibited by anti–IL-5 antibody (61,63). IL-2, however, has been demonstrated to be a potent chemotactant of eosinophils (38,62), and T lymphocytes from patients with eosinophilia produce IL-5 after stimulation with IL-2 (64). These data suggest that IL-2 production might indirectly stimulate the production and activation of eosinophils.

Our results demonstrate an association of eosinophilia in chronic HD patients with increased cytokine responsiveness during HD with cuprammonium membranes. This suggests that IL-2 and associated IL-5 production may be etiologic factors in the pathogenesis of HD-associated eosinophilia. This hypothesis is supported by the clinical observation that the use of polysulfone membranes, which generally do not result in cytokine activation (52), improved dialysis-associated eosinophilia (65,66). Also, the ability of cyclosporin A to diminish eosinophilia in patients with atopic dermatitis (67) and in an animal model (68) may be because of the suppression of cytokine production (69). Atopic individuals demonstrate a deficiency of interferon gamma and increased IL-5 levels (42), resulting in eosinophilia, and interferon gamma opposes the stimulatory effect of IL-5 on eosinophils (70).

Eosinophilia in HD patients has been associated with atopy, as manifested by elevated total circulating IgE levels and the presence of specific IgE directed against various HD-associated allergens such as ethylene oxide gas, isocyanates, formaldehyde, and phthalic anhydride (5,29,71-73). Eosinophilia in patients treated with chronic peritoneal dialysis has been associated with hypersensitivity to tubing material and ethylene oxide gas (28,72,74,75). Recent studies have examined the role of cytokines in the pathogenesis of atopy and allergic reactions (39-42,76,77). IL-2 causes B-cell proliferation and the production of antibodies, including IgE (41,78). IgE synthesis is primarily stimulated by IL-4 and suppressed by interferon gamma (79-81). Both normal and atopic patients produce IL-2, IL-4, and IL-5 concomitantly (39,42,82), but atopic subjects have exaggerated IL and diminished interferon production (42). Exaggerated IgE synthesis and eosinophilia therefore result in atopic patients (79-81,61,63). The administration of recombinant IL-2 is associated with both IL-5 and IL-4 elevation in many patients (83). Thus, in predisposed individuals, exaggerated cytokine production during HD might lead to IgE production against specific dialysis-associated allergens. On the other hand, allergen-antibody interaction may lead to the release of IL-1 and IL-2 at the site of allergen challenge (43,44,84). However, it is not clear whether cytokine production is the cause or the result of allergy.

Many studies have addressed the influence of dialysis-related factors on cytokine production, but patient factors have not been evaluated. We found that chronic HD patients could be classified as responders or nonresponders in terms of their stimulated cellular cytokine production. Exaggerated HD-associated cytokine production may depend on the presence of atopy and may play a role in the pathogenesis of HD-associated eosinophilia and hypersensitivity. In contrast to the measurement of IL, eosinophil counts are readily obtainable and may be a surrogate marker for the presence of exaggerated cellular cytokine production during HD. Clearly, further studies are needed to improve our understanding of the role of cytokines in patients with renal disease.

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