Eosinophilic pleural effusions (EPEs), which are defined as those containing at least 10% eosinophils, are due to a variety of inflammatory and malignant pleural diseases. The most common etiology is mechanical pleural injury resulting in the presence of air and/or blood in the pleural cavity.\textsuperscript{1} Although EPEs are frequently encountered in clinical practice, few studies have focused on the pathogenesis of pleural fluid (PF) eosinophilia.\textsuperscript{1} Post-coronary artery bypass grafting (CABG) pleural effusions, especially those occurring within the first
month after the procedure, are probably due to mechanical irritation of the pleura during surgery and are frequently eosinophilic. Thus, we believe that post-CABG pleural effusions represent an interesting clinical model with which to study the pathogenesis of EPE associated with mechanical pleural injury.

The cascade of events that leads to tissue accumulation of eosinophils includes the increased production of eosinophils in the bone marrow, the migration of eosinophils to the tissues through the bloodstream, and the extended survival of the cells in the tissues. Interleukin (IL)-5, IL-3, and granulocyte-macrophage colony-stimulating factor stimulate eosinophilopoiesis in bone marrow, and suppress eosinophil apoptosis at the site of inflammation. Transmigration into tissues is initiated by various eosinophil chemoattractants and is mediated by adhesion molecules that are expressed on the surface of endothelial cells and eosinophils. Vascular cell adhesion molecule (VCAM)-1 is an adhesion molecule that is expressed by the endothelial cells, and mediates the rolling and the firm adhesion of the eosinophils on the endothelial surfaces, augmenting the tissue accumulation of these cells. We have previously shown that IL-5 and VCAM-1 levels are higher in EPEs than in non-EPEs, and that their PF levels correlate with the number and percentages of PF eosinophils. However, in the previous studies we could not determine whether these molecules are produced locally in the pleura or their increased levels are due to high serum levels. Regulated on activation, normal T-cell expressed and secreted and eotaxin are very potent chemoattractants for the eosinophils. However, in a previous study we could not detect any significant difference between regulated on activation, normal T-cell expressed and secreted, and eotaxin levels in EPEs and non-EPEs, an observation suggesting that other eosinophil chemoattractants are involved in the pathogenesis of EPE. Two possible candidates are eotaxin-2 and eotaxin-3, which are potent chemoattractants for the eosinophils and share the same receptor with eotaxin-1.

In the present study, we examined the relationship between PF and peripheral blood eosinophilia in patients with post-CABG pleural effusions. We also measured the PF and the corresponding serum levels of IL-5, eotaxin-2, eotaxin-3, and VCAM-1. We hypothesized the following: (1) the number of PF eosinophils would correlate with the number of the blood eosinophils; (2) the PF levels of the examined cytokines and VCAM-1 would be higher in EPEs than in post-CABG non-EPEs; (3) the PF levels of the examined cytokines and VCAM-1 would correlate with the number and the percentage of the PF eosinophils; and (4) in patients with post-CABG EPEs, the PF levels of the cytokines and VCAM-1 would be higher than the corresponding serum levels.

Materials and Methods

The study was approved by the Institutional Review Board at St. Thomas Hospital, and every patient signed an informed consent form. Thirty-eight patients who had undergone coronary artery bypass surgery within the previous month and had undergone thoracocentesis for a pleural effusion were prospectively recruited at St. Thomas Hospital between December 2002 and September 2003. Blood was drawn immediately after thoracocentesis. PF and blood samples were centrifuged. The supernatants and the serum samples were stored at −80°C. PF RBC and nucleated cell counts were obtained by manual microscopy. The differential nucleated cell counts of the PF samples were obtained by manually counting 100 cells on a Wright-stained smear after the cells had been concentrated by cytocentrifugation at 2000 revolutions per minute for 10 min. The peripheral blood WBC count and the differential cell counts were performed by an automated counter (Coulter Electronics; Lutton, UK). The differential cell count was determined by direct microscopic examination of slides stained with a modified Wright-Giemsa stain. A pleural effusion was called an EPE when it contained at least 10% eosinophils. Peripheral blood eosinophilia was defined as the presence of at least 400 eosinophils/μL. The subject’s medical records were reviewed by a pulmonologist to confirm the diagnosis of post-CABG pleural effusion. Enzyme-linked immunosorbent assay was performed (Quantikine enzyme-linked immunosorbent assay kits; R&D Systems Inc; Minneapolis, MN) to test for the following: IL-5 (minimum detectable dose, <3 pg/mL); eotaxin-2 (minimum detectable dose, <1.83 pg/mL); eotaxin-3 (minimum detectable dose, <2.33 pg/mL); and VCAM-1 (minimum detectable dose, <2 ng/mL).

Statistical Analysis

Values were reported as the mean ± SEM when they were normally distributed and as the median (interquartile range [IQR]) when they were not normally distributed. To assess the difference in the frequency of blood eosinophilia between patients with EPEs and those with non-EPEs, the Fisher exact test was used. To assess the difference between EPEs and non-EPEs for one variable, the independent samples t test or the Mann-Whitney test was used, as appropriate. To assess the difference between a PF variable and the corresponding serum variable, the Wilcoxon signed rank test was used because values were not normally distributed. To assess the correlation between two variables, the Spearman test was used because values were found not to be normally distributed. For statistical analysis and the construction of figures, a statistical software package (SPSS, version 11.0; SPSS Inc; Chicago, IL) was used.

Results

PF and Blood Eosinophilia

Thirty-eight patients with post-CABG pleural effusions were included in the study (unilateral pleural effusions, 36 patients; bilateral pleural effusions, 2
patients; total No. of pleural effusions, 40). An EPE was found in 13 of the 38 patients (in 11 of the 36 patients with unilateral pleural effusions, and in both patients with bilateral pleural effusions). The first patient with a bilateral pleural effusion had EPEs on both sides (50% eosinophils in one hemithorax and 29% in the other). The second patient had an EPE on one side (47% eosinophils), while there were no PF eosinophils in the contralateral hemithorax. Thus, 14 of the total of 40 pleural effusions were EPEs. The median percentage of PF eosinophils was 35.5% (IQR, 27.3%; range, 12 to 82%) in EPEs and 0% (IQR, 1.25%; range, 0 to 8%) in non-EPEs. The per-
centage of blood eosinophils in patients with EPEs was lower than the corresponding blood eosinophils was 588 cells/μL (IQR, 379 cells/μL) in patients with non-EPEs (p = 0.78). The PF RBC counts did not correlate with the number (p = 0.49) or the percentage (p = 0.73) of PF eosinophils.

Patients with EPEs had more peripheral blood eosinophils (both in percentage and absolute number) than patients with non-EPEs. The mean percentage of blood eosinophils was 7 ± 1.3% (range, 0 to 16%) in patients with EPEs and 2.6 ± 0.3% (range, 0 to 6%) in patients with non-EPEs (p < 0.001). Similarly, the median number of blood eosinophils was 588 cells/μL (IQR, 832 cells/μL) in patients with EPEs and 195 cells/μL (IQR, 379 cells/μL) in patients with non-EPEs (p < 0.001). Peripheral blood eosinophilia was significantly more common in patients with EPEs than in those with non-EPEs (p = 0.003) [Table 1]. The blood eosino-
phil percentage was lower than the corresponding blood eosinophil percentage in every patient with a unilateral EPE. The percentage and the number of PF eosinophils correlated significantly with the per-
centage of blood eosinophils (r = 0.54; p < 0.001) and the number of blood eosinophils (r = 0.52; p = 0.001), respectively.

**PF and Serum Levels of Cytokines and VCAM-1**

Detectable levels of eotaxin-2 and VCAM-1 were present in every PF and serum sample. Detectable levels of IL-5 were present in every PF sample but in only nine serum samples (24%), eight (62%) from patients with EPEs and 1 (4%) from a patient with a non-EPE. Detectable levels of eotaxin-3 were present in every eosinophilic PF sample and in 17 of the noneosinophilic PF samples (65%). Serum levels of eotaxin-3 were detectable in only 11 patients (29%), 4 of whom (31%) had EPEs and 7 of whom (28%) had non-EPEs. The results of the measure-
ments in PF and serum are shown in Table 2.

**Table 1—Frequency of Blood Eosinophilia in Patients With EPEs and non-EPEs***

<table>
<thead>
<tr>
<th>Pleural Effusions</th>
<th>Blood Eosinophilia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Non-EPEs</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>EPEs</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>14</td>
</tr>
</tbody>
</table>

*p < 0.001 for non-EPEs vs EPEs.

The median PF IL-5 level was significantly higher than the median serum levels in all patients (p < 0.001), in patients with EPEs (p = 0.001), and in patients with non-EPEs (p < 0.001). The PF IL-5 concentrations were higher than the corresponding serum concentrations in every patient in our study. The median PF IL-5 level was significantly higher in EPEs than in non-EPEs (p < 0.001). The median serum IL-5 level was significantly higher in patients with EPEs than in those with non-EPEs (p = 0.002). The median PF eotaxin-2 level was significantly lower than the corresponding serum level in all of the pleural effusions (p < 0.001) and in non-EPEs (p < 0.001). The median PF and serum eotaxin-2 levels did not differ significantly between EPEs and non-EPEs.

The median PF eotaxin-3 levels were significantly higher than the corresponding serum levels in all patients (p < 0.001), in patients with EPEs (p = 0.009), and in patients with non-EPEs (p = 0.02). The median PF eotaxin-3 level but not the median serum eotaxin-3 level was significantly higher in EPEs than in non-EPEs (p = 0.001).

The median PF VCAM-1 level was significantly lower than the corresponding serum level in all of the pleural effusions (p = 0.001) and in non-EPEs (p = 0.004), but it did not differ significantly in EPEs. The median PF but not the serum VCAM-1 level was significantly higher in EPEs than in non-
EPEs (p = 0.02).

**Correlations Between PF and Serum Levels of Cytokines and VCAM-1**

PF IL-5 levels significantly correlated with the serum levels of the cytokine (r = 0.5; p = 0.001). PF eotaxin-2 levels correlated significantly with the serum levels (r = 0.34; p = 0.03). There was no signif-
icient correlation between PF levels of eotaxin-3 or VCAM-1 and the corresponding serum levels.

**Correlations Between Levels of Cytokines, and Levels of VCAM-1 and Eosinophils**

In the PF, PF IL-5 levels significantly correlated with the number of PF eosinophils (r = 0.7;
p < 0.001) and the percentage of PF eosinophils (r = 0.69; p < 0.001) [Fig 1]. Similarly, PF eotaxin-3 levels correlated significantly with the number of PF eosinophils (r = 0.5; p = 0.001) and the percentage of PF eosinophils (r = 0.48; p = 0.002) [Fig 2]. PF levels of eotaxin-2 and VCAM-1 did not correlate significantly with the number or the percentage of PF eosinophils.

In the Blood: Serum IL-5 levels significantly correlated with the number of blood eosinophils (r = 0.54, p < 0.001) and the percentage of blood eosinophils (r = 0.46; p = 0.003). Serum levels of eotaxin-2, eotaxin-3, and VCAM-1 did not correlate significantly with the number or the percentage of blood eosinophils.

**Discussion**

In the present study, we examined the PF and blood eosinophilia in patients with post-CABG EPEs and non-EPEs, and their possible association with PF and blood levels of IL-5, eotaxin-2, eotaxin-3, and VCAM-1. Our main findings were as follows: (1) the percentage and the number of PF eosinophils correlated significantly with the percentage and the number of blood eosinophils, respectively; (2) PF IL-5 levels were significantly higher than the corresponding serum IL-5 levels in every patient with a post-CABG pleural effusion (there was a significant correlation between the PF and serum IL-5 levels), and PF and serum IL-5 levels correlated significantly with the number and the percentage of PF and blood eosinophils, respectively; (3) PF eotaxin-3 levels were significantly higher than the corresponding serum levels, but there was no significant correlation between PF and serum eotaxin-3 levels, with PF eotaxin-3 levels correlating significantly with the number and the percentage of PF eosinophils; (4) PF eotaxin-2 levels were significantly lower than the corresponding serum levels, and there was a significant correlation between the PF levels and the corresponding serum levels, with PF or serum eotaxin-2 levels not differing significantly between patients with EPEs and those with non-EPEs (no correlation between PF eotaxin-2 levels and PF eosinophil levels); and (5) PF VCAM-1 levels were significantly lower than the corresponding serum levels, and there was no significant correlation between PF and serum levels, with PF VCAM-1 levels being significantly higher in EPEs than in non-

---

**Table 2—PF and serum levels of IL-5, Eotaxin-2, Eotaxin-3, and VCAM-1 in EPEs and non-EPEs**

<table>
<thead>
<tr>
<th>Variables</th>
<th>All</th>
<th>EPE</th>
<th>Non-EPE</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5, pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>34.5 (142)†</td>
<td>286 (385)†</td>
<td>14 (31)†</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum</td>
<td>0 (0.75)</td>
<td>1 (2.25)</td>
<td>0 (0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Eotaxin-2, pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>59 (58)†</td>
<td>74.5 (202)</td>
<td>57 (53)†</td>
<td>NS</td>
</tr>
<tr>
<td>Serum</td>
<td>250 (231)</td>
<td>250 (230)</td>
<td>275.5 (242)</td>
<td>NS</td>
</tr>
<tr>
<td>Eotaxin-3, pg/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>3.5 (19.8)†</td>
<td>18.5 (56)†</td>
<td>2 (6.5)†</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum</td>
<td>0 (1)</td>
<td>0 (2.25)</td>
<td>0 (0.25)</td>
<td>NS</td>
</tr>
<tr>
<td>VCAM-1, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>542 (227)†</td>
<td>621 (155)</td>
<td>496 (217)†</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum</td>
<td>703 (548)</td>
<td>679 (378)</td>
<td>727.5 (653)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values given as median (IQR), unless otherwise indicated. NS = not significant.
†EPEs vs non-EPEs.
‡Significantly different (p < 0.05) than corresponding serum values.
Peripheral blood eosinophilia is a common finding in patients with EPEs of different etiologies\(^1\), but it is not always present in patients with mechanical pleural injury.\(^7,8\) Our findings suggest a strong association between PF and peripheral blood eosinophilia in patients with post-CABG EPEs. In agreement with previous observations in posttraumatic EPEs,\(^9,10\) the eosinophil percentage in the PF was always higher than that in the blood. This observation probably reflects the fact that eosinophils, produced in the bone marrow, have a very short half-life in the blood, being rapidly attracted into the pleural space. The absence of peripheral blood eosinophils in a minority of patients with post-CABG EPEs may indicate that the eosinophilic reaction had started to regress in these patients. The clinical implication of the above findings is that the presence of blood eosinophilia in a patient with a post-CABG pleural effusion is suggestive of PF eosinophilia. However, blood eosinophilia should not be considered as an absolute indicator for PF eosinophilia, since it was not observed in every patient with an EPE, and it was also found in a minority of patients (4 of 25 patients) with a non-EPE.

In the present study, we examined for the first time both PF and serum concentrations of different proteins that are known to contribute to eosinophilic inflammation in patients with EPEs and non-EPEs. Our results indicate a strong association between IL-5 and the eosinophilic reaction in response to pleural injury induced by CABG surgery. We showed that PF IL-5 levels were significantly higher in patients with post-CABG EPEs than in those with post-CABG non-EPEs, and that PF IL-5 levels correlated significantly with the number and the percentage of PF eosinophils. This finding confirms previous observations\(^4,10–12\) concerning the role of IL-5 in the pathogenesis of EPEs of different etiologies.

The present study demonstrates that the median PF IL-5 level was significantly higher than the corresponding serum level, and that there was a significant correlation between the PF and serum IL-5 levels. This observation suggests that IL-5 is produced in the pleural cavity and that a portion of the cytokine enters the bloodstream. The notion of the local production of IL-5 in the pleural space was initially raised by Schandene and coworkers,\(^10\) who reported that IL-5 was produced by PF but not blood CD4\(^+\) lymphocytes in three patients with posttraumatic EPEs. Our study not only confirms in a larger number of patients that IL-5 is produced locally in the pleural compartment in EPEs associated with mechanical pleural injury, but also shows for the first time that PF levels of this cytokine are higher than the corresponding serum levels, even in patients with post-CABG non-EPEs. Accordingly, one can speculate that pleural IL-5 production is commonly induced by CABG surgery but that it does not always lead to the accumulation of eosinophils in the pleural cavity, suggesting that other cytokines and adhesion molecules are necessary for the development of postsurgical EPEs.

We also observed that serum IL-5 levels were significantly higher in patients with EPEs than in those with non-EPEs, and that they correlated significantly with the number and the percentage of blood eosinophils, a finding that implies a role for serum IL-5 in the pathogenesis of pleural eosinophilia. Thus, combining the observations made of PF and serum, we could speculate that mechanical irritation of the pleura caused by surgery induces the production of IL-5 in the pleural cavity, followed by a spill of the cytokine into the circulation and subsequent stimulation of eosinophilopoiesis in the bone marrow. As a result, the number of circulating eosinophils increased. Eosinophil chemoattractants and adhesion molecules are then required to complete the translocation of the eosinophils from the blood to the pleural cavity.

Chemokines are cytokines with chemotactic activity for inflammatory cells. Among them, the eotaxins (\(1, 2, \) and \(3\)) are potent eosinophil chemoattractants and activators, and they participate in the

---

**Figure 2.** Correlation between PF eotaxin-3 levels and PF eosinophil levels. \(\bigcirc\) = EPEs; \(\triangle\) = non-EPEs.
pathogenesis of eosinophilic human diseases.\textsuperscript{5,6} Moreover, some data\textsuperscript{13} have suggested that they may also prolong eosinophil survival. Yokoyama and co-workers\textsuperscript{14} reported that PF eotaxin-1 (CCL-11) levels were higher in EPEs than in non-EPEs and that they correlated with the number of PF eosinophils. However, this finding was not confirmed by a previous study performed by our group.\textsuperscript{4}

In the present study, we examined the levels of eotaxin-2 (CCL-24) and eotaxin-3 (CCL-26), which share the same receptor with eotaxin-1 and have the same effects on eosinophils as eotaxin-1. This is the first report on eotaxin-2 and eotaxin-3 levels in pleural effusions. PF eotaxin-3 levels were significantly higher than the matched serum levels, suggesting that eotaxin-3 is produced locally in the pleural space in patients with post-CABG pleural effusions. PF eotaxin-3 levels were significantly higher in EPEs than in non-EPEs and correlated with the number of the PF eosinophils, findings that suggest that eotaxin-3 is involved in EPE pathogenesis. The low concentration of eotaxin-3 in serum and the absence of any significant difference in serum levels between patients with EPEs and those with non-EPEs suggests that increased serum levels of eotaxin-3 are not important for eosinophil recruitment in the pleural space and that the chemotactic activity of this chemokine is restricted to eosinophils passing through the pleural vasculature. In agreement with our assumption that eotaxin-3 participates in the pleural localization of eosinophils, Cuvelier and Patel\textsuperscript{15} showed that the transmigration of eosinophils on activated endothelial cells is regulated by eotaxin-3.

PF levels of eotaxin-2 were significantly lower than its corresponding serum levels, and there was a significant correlation between the PF levels of the chemokine and the corresponding serum levels. These findings suggest that eotaxin-2 is not preferentially produced in the pleural cavity and that its presence in the PF may be the result of diffusion from the blood. Moreover, our findings imply that eotaxin-2 does not have a role in the pathogenesis of post-CABG EPEs, since its PF levels did not differ between patients with EPEs and those with non-EPEs, and did not correlate with the PF eosinophil counts or percentages. In agreement with our findings, other investigators have reported that eotaxin-3 is more important than eotaxin-2 in the tissue eosinophil accumulation that occurs in patients with allergic diseases.\textsuperscript{10,17} VCAM-1 is an adhesion molecule that is expressed on the surface of endothelial cells and mediates the recruitment of eosinophils at sites of inflammation.\textsuperscript{3} We have previously reported that PF VCAM-1 levels are higher in EPEs than in non-EPEs, and that they correlate with the number and the percentage of PF eosinophils. One of the questions raised from this previous study was whether VCAM-1 is locally produced in the pleural cavity. The results of the present study do not support this possibility since the serum VCAM-1 levels were significantly higher than the PF VCAM-1 levels. Although we confirmed that the PF VCAM-1 levels were higher in EPEs than in non-EPEs, we did not find a significant correlation between PF VCAM-1 levels and either the number or the percentage of PF eosinophils in the present study. Hence, although it is likely that VCAM-1 mediates the transmigration of eosinophils into the pleural cavity, its importance in the production of EPEs remains to be elucidated.

It is interesting to note that a minority of post-CABG pleural effusions included in the present study were eosinophilic. This could be explained by the timing of the development of the pleural effusions included in the study since previous observations\textsuperscript{18} have suggested that pleural effusions occurring later than the first month after surgery usually do not contain increased numbers of eosinophils. However, this explanation is not likely since, in the present study, EPEs were not found to occur earlier than non-EPEs and the median latent time of both was <30 days. PF eosinophilia could be also attributed to the bloody nature of some effusions. Again, this assumption was not supported by the results of the present study since PF eosinophil levels did not correlate with the PF RBC count. Thus, it is unclear why some post-CABG pleural effusions are eosinophilic while others are not. This phenomenon can be due to differences in the severity of pleural injury during the operation or may represent different individual responses that are influenced by genetic factors. Whatever the underlying mechanism, the present study strongly suggests that IL-5 and eotaxin-3 are involved in the pathogenesis of post-CABG-associated PF eosinophilia.

In conclusion, our results indicate that in patients with post-CABG pleural effusions, IL-5 and eotaxin-3 are produced in the pleural cavity, and participate in the pathogenesis of PF eosinophilia. Furthermore, it is likely that increased serum IL-5 levels result from an overflow from the pleural compartment, and may potentially stimulate increased production and mobilization of eosinophils from the bone marrow and their accumulation in the PF. VCAM-1 levels are elevated in EPEs and may participate in EPE pathogenesis. We also showed that in a patient with post-CABG pleural effusions, the presence of peripheral blood eosinophilia suggests that the effusions are eosinophilic.
REFERENCES
4 Kalomenidis I, H Mohamed KH, Lane KB, et al. Pleural fluid levels of vascular cell adhesion molecule-1 are elevated in eosinophilic pleural effusions. Chest 2003; 124:159–166
13 Shinagawa K, Trifileff A, Anderson GP. Involvement of CCR3-reactive chemokines in eosinophil survival. Int Arch Allergy Immunol 2003; 130:150–157