RAPID COMMUNICATION

Presence of Autoantibodies to Interleukin-8 or Neutrophil-Activating Peptide-2 in Patients With Heparin-Associated Thrombocytopenia


Eighty-seven patients with heparin-associated thrombocytopenia (HAT) showed either a positive hepatic platelet aggreometry test result and/or the presence of antibodies to heparin-platelet factor 4 (HPF4) complexes by enzyme-linked immunosorbent assay (ELISA). Fifteen of these patients lacked antibodies to HPF4, and plasma from these patients was analyzed for the presence of antibodies to PF4-related chemokines, Neutrophil-activating peptide-2 (NAP-2) and interleukin-8 (IL-8). Of these 15 patients, 6 showed antibodies to IL-8 and 3 to the platelet basic protein (PBP)-derived protein, NAP-2. Antibodies to IL-8 and NAP-2 were not observed in control patients (n = 38), patients with HAT and HPF4 autoantibodies (n = 72), patients with autoimmune diseases (n = 21), or patients with non-HAT thrombocytopenia (n = 30). Five of these nine patients with anti-IL-8 or anti-NAP-2 developed thrombosis during hepatic treatment, which is not statistically different from the patients with HPF4 antibodies. The existence of autoantibodies to IL-8 and NAP-2 in HAT patients highlights the significance of chemokines in the pathogenesis of HAT. The contribution of heparin in vitro was minimal in patients with anti-IL-8 and anti-NAP-2 antibodies, suggesting a biologic difference from the majority of patients with HAT and anti-HPF4 antibodies. It may be that antibodies to IL-8 and NAP-2 have weaker affinity for heparin and that the ELISA system may not reflect in vivo heparin-chemokine complex formation. Alternatively, antichemokine autoantibodies may predetermine heparin exposure, and the role of heparin in initiating HAT may be to mobilize the chemokines and to target them to platelets, neutrophils, or endothelial cells. Subsequent chemokine-binding autoantibodies then lead to cell activation resulting in thrombocytopenia and thrombosis.

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SEVERE AUTOIMMUNE heparin-associated thrombocytopenia (HAT) is a major life-threatening complication of heparin therapy associated with thrombocytopenia and sometimes thrombosis.1-4 This iatrogenic disorder has been shown to involve antibodies directed to a complex of heparin and platelet factor 4 (PF4),5 a chemokine of the C-X-C family.6 Our initial studies on the role of heparin-PF4 (H-PF4) immune complexes in HAT have recently been confirmed.7-10 To measure antibodies to H-PF4 complexes, an enzyme-linked immunosorbent assay (ELISA) technique has been designed11 that has been extensively used for the diagnosis of patients with HAT.12,13 The H-PF4 immune complexes can bind to platelet membranes12 and to endothelial cells.9 When the antibody involved is an IgG, the Fc portion can bind to the platelet FcγRIIA receptor, inducing platelet activation, aggregation, and possibly thrombosis.8,10 We have recently reported that HAT can also be induced by IgM and/or IgA antibody isotypes.13 Thrombocytopenia and platelet activation may then be directly produced by antibody binding or mediated through Fc(γ)R receptors on lymphocytes,14 Fc(α)R receptors on neutrophils and monocytes,15,16 or through complement activation for IgM isotypes.

Antibodies to H-PF4 complexes are not detected in about 15% of HAT patients documented by thrombocytopenia and platelet activation studies.17 We have therefore investigated whether other related chemokines present at the site of vessel injury could be involved in this subgroup of patients. Our studies first focused on the platelet basic protein (PBP)-derived chemokines that, like PF4, are expressed at high levels in megakaryocytes and stored in platelet α-granules. The 94 amino-acid (aa) PBP is found in megakaryocytes and has about 40% aa homology with PF4. Mature IL-8 is released as a 77-aa protein by endothelial cells and is a 77-aa N-terminally shorter form by monocytes and neutrophils.21 IL-8, which binds to heparin, although with a lower affinity than PF4 or NAP-2, is a potent chemoattractant and activator of neutrophils, binding to these.5
cells through both the IL-8 receptors, type A and B.\textsuperscript{20,21} IL-8 is also an angiogenic agent and endothelial cells and platelets have been reported to express IL-8 receptors.\textsuperscript{22,24,25}

In this report, we have investigated whether autoantibodies to NAP-2 or IL-8 could be detected in patients with HAT. We found that antibodies to IL-8 or to NAP-2 are present in more than half of 15 patients with HAT showing positive platelet aggregometry test results but no antibodies to H-PF4, demonstrating the existence of other autoantibodies in this subgroup.

**PATIENTS, MATERIALS, AND METHODS**

*Reagents and chemicals.* Chemokines used for ELISA assays were prepared as follows. Recombinant (r) PBP, CTAP-III, β-TG, NAP-2, and PF4 were expressed in bacteria and isolated using a heparin agarose column followed by high pressure liquid chromatography, as previously described.\textsuperscript{26,27} Commercial r-NAP-2 was provided by Bachem (Basel, Switzerland). Native β-TG\textsuperscript{28} and PF4\textsuperscript{29} were prepared from human platelets as previously described. r-IL-8 (72-aa form) was purchased from R&D Systems (Abingdon, UK) and from Immunogenetics (Anwerpen, Belgium). Chemically activated Covalink micro-ELISA plates were supplied by Nunc (Roskilde, Denmark). Ortho-phenylene-diamine and hydrogen peroxide were from Sigma (St Louis, MO), m-Maleimido-benzoyl-N-hydroxy-sulfo-succinimide (MBSS) was supplied by Pierce (Interchim, Paris, France). All other chemicals used were of analytical grade and provided by Prolabo (Paris, France). The second antibody specific for the various human Ig isotypes (IgG, IgA, or IgM) was supplied by Biosys (Compiègne, France) and was coupled to peroxidase using the periodate oxidation method.\textsuperscript{30} Anti-IgG,A,M peroxidase or anti-IgG, anti-IgA, and anti-IgM peroxidase conjugates were prepared as previously reported.\textsuperscript{31} The anti-IgG,A,M peroxidase was prepared to offer a homogeneous reactivity regardless of the isotype tested. To achieve this goal, specific peroxidase conjugates for IgG (γ-specific), IgA (α-specific), and IgM (μ-specific) were prepared; their reactivity was standardized for binding to IgG-, IgA-, or IgM-coated plates; and they were mixed together for obtaining the global conjugate. Antibody isotypes were determined using the monovalent anti-IgG, anti-IgA, and anti-IgM peroxidase conjugates instead of the mixture.

**Heparin-PF4 and chemokine ELISAs.** Assay of antibodies to PF4 and H-PF4 complexes was performed as previously described\textsuperscript{10}, using 200 μL of a mixture of 20 μg/mL of PF4 and 0.5 IU/mL of heparin (Celsius, Cincinnati, OH) per microwell. The positive range was defined for absorbance values at 492 nm (A492) above that of the mean value obtained for control groups + 3 standard deviations (SD). When the assay is performed at room temperature (RT), the reactivity range for positive samples corresponds to an A492 > 0.5. Assays of antibodies to NAP-2 and its precursors (β-TG, CTAP-III, and PBP) and to IL-8 were performed using the same methodology. The latter chemokines were coated into microwells of the MBSS-pretreated covalink plates as follows: 200 μL of a 125 mg/L solution of MBSS was introduced into the microwells and incubated for 4 hours at RT. Plates were washed and 200 μL of a 0.1 mol/L sodium phosphate buffer, pH 7.5, containing 20 μg/mL of the chemokine, with or without 0.1 to 2 IU/mL heparin, were introduced per well. The plates were incubated overnight at RT. Unbound material was washed away and the plates were then incubated with 10% goat serum in coating buffer for 2 hours at RT. Control plates without any chemokine were similarly prepared. Detection of antibodies was performed using 1:100 dilutions of plasma samples in 0.05 mol/L phosphate buffer, 0.15 mol/L NaCl, and 10% goat serum, pH 7.5 (dilution buffer). Test samples (200 μL) were incubated for 1 hour at RT. The plates were then washed with 0.025 mol/L phosphate buffer.

![Fig 1. Assay of antibodies to H-PF4 complexes in 87 patients with HAT by ELISA. Seventy-two patients were positive ( []) for H-PF4 complexes, with 18 of them showing negative platelet aggregometry test results ( ). It is noteworthy that 15 patients with positive platelet aggregometry test results had no antibodies to H-PF4.]
buffer, 0.15 NaCl, 0.1% Tween-20, pH 7.5. The wells were then incubated with the global anti-IgG,A,M peroxidase conjugate (200 μL per well) in dilution buffer. After washing, color development was performed with OPD/H₂O₂ for 5 minutes at RT before stopping the reaction with 3 mol/L H₂SO₄. Absorbance was measured at 492 nm within 30 minutes of stopping the reaction. The isotyping was similarly performed with the monospecific conjugates instead of the global conjugate. All the assays were performed at RT and positive specimens were systematically used as internal controls in each series to check the assay reproducibility.

**Blood collection.** Blood was collected using Na₂ EDTA anticoagulant for platelet counting. For all the other laboratory tests, blood was collected using the conventional citrate anticoagulant (9 vol blood per 1 vol 0.109 mol/L trisodium citrate) and centrifuged for 20 minutes at 3,000g. Citrated plasma supernatant was decanted, aliquoted, and stored frozen at -80°C. Just before use, samples were thawed for 15 minutes at 37°C.

**Platelet studies.** Platelet aggregometry studies were performed with heparin (Calciparin; Sanofi-Choay, Paris, France) at 0.5 and 1 IU/mL, as previously described.³³

**Study population.** Eighty-seven patients under heparin therapy with unfractionated heparin (Calciparin; Sanofi-Choay) or low molecular weight heparin (LMWH; Fraxiparin; Sanofi-Choay) for preventive (surgery or thrombotic antecedents) or curative (venous or arterial thrombosis) indications had a clinical diagnosis of HAT. They presented with a severe platelet decrease (<100 x 10⁹/L or a decrease >30% of the initial platelet count) occurring at least 5 days after the onset of heparin therapy (earlier when there was a previous exposure to this drug), associated with a correction after heparin withdrawal. Patients are described in Table I. All patients had a positive platelet aggregometry test result in the presence of heparin and/or developed antibodies to H-PF4. Twenty-four (27.5%) patients presented with significant thrombotic complications during heparin therapy. In 3 cases, there was only a slight and progressive increase of platelet count after the withdrawal of heparin. Eight patients developed late thrombocytopenia, occurring 15 to 26 days after onset of treatment. Heparin therapy was monitored using activated partial thromboplastin time (APTT) and specific measurement of plasma heparin concentrations with an anti-Xa assay (Staclot Heparin; Diagnostica Stago, Asnières, France). All patients were analyzed retrospectively for the presence of antibodies to H-PF4 complexes. Seventy-two patients were found positive for these autoantibodies.
TABLE 2. Clinical Characteristics and Platelet Count of the 9 Patients With HAT Showing Antibodies to IL-8 or to NAP-2

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>Therapy</th>
<th>Interval* (d)</th>
<th>Platelet Count (10^9/L)</th>
<th>Platelet Aggregation Test</th>
<th>Specificity of Antibodies</th>
<th>Isotype</th>
<th>Clinical Status Before Heparin Therapy</th>
<th>Thrombotic Complications Under Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64/F</td>
<td>LMWH</td>
<td>7</td>
<td>178 56</td>
<td>+</td>
<td>IL-8</td>
<td>IgM</td>
<td>Malignancy</td>
<td>Leg thrombosis leukemia</td>
</tr>
<tr>
<td>2</td>
<td>91/F</td>
<td>LMWH</td>
<td>14</td>
<td>275 40</td>
<td>+</td>
<td>IL-8</td>
<td>IgG</td>
<td>Hip replacement</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>64/M</td>
<td>UH</td>
<td>19</td>
<td>71 62</td>
<td>+</td>
<td>NAP-2</td>
<td>IgM</td>
<td>Mitral valve replacement</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>49/M</td>
<td>UH</td>
<td>15</td>
<td>279 50</td>
<td>+</td>
<td>NAP-2</td>
<td>IgG</td>
<td>Hip replacement</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>5</td>
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<td>6</td>
<td>560 87</td>
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<td>IL-8</td>
<td>IgG, IgM</td>
<td>Autoimmune disease</td>
<td>Thrombosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peripheral vascular disease</td>
<td>Amputation</td>
</tr>
<tr>
<td>6</td>
<td>68/M</td>
<td>LMWH</td>
<td>11</td>
<td>240 58</td>
<td>+</td>
<td>IL-8</td>
<td>IgG</td>
<td>Infection—</td>
<td>Thrombosis</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Heart surgery</td>
<td>Amputation</td>
</tr>
<tr>
<td>7</td>
<td>34/M</td>
<td>UH</td>
<td>21</td>
<td>170 74</td>
<td>+</td>
<td>NAP-2</td>
<td>IgG, IgM</td>
<td>Malignancy</td>
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<tr>
<td>8</td>
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<td>IL-8</td>
<td>IgG</td>
<td>Cholecystectomy—</td>
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<tr>
<td>9</td>
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<td>6</td>
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<td>IL-8</td>
<td>IgG</td>
<td>Malignancy—</td>
<td>Pulmonary embolism</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bone fracture</td>
<td></td>
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</tbody>
</table>

* Interval between initiation of heparin and occurrence of thrombocytopenia.
† Platelet count at day 0 (before heparin treatment) and day X (platelet nadir).

including 18 with negative platelet aggregation tests. Fifteen patients showed positive aggregometry tests but lacked autoantibodies to H-PF4. Twelve of these 15 patients had typical symptoms of HAT. The last 3 patients were atypical: their platelet counts were already low before heparin therapy, decreased during treatment, and only progressively increased after heparin withdrawal. Six of the 15 patients (40%) developed thrombosis while receiving heparin.

Statistical analysis. Statistical analysis was performed using the Statistical Analysis System (SAS) software (SAS Institute Inc, Cary, NC). Mean values and standard deviations were calculated. Comparison between groups and incidence of pathological events was analyzed using the Fischer's test, the λ- and U-tests, or the t-test Cochran for quantitative values. The χ² test did not apply because of the too small sample size of the IL-8 and NAP-2 groups.

RESULTS

Assay of antibodies to H-PF4 and platelet aggregometry tests. Patients with HAT were retrospectively analyzed for the presence of autoantibodies to H-PF4 (Fig 1). The H-PF4 ELISA test showed that 72 of the 87 HAT patients had antibodies to H-PF4, with A492 > 0.5. As in previous studies, no normal subjects nor patients with thrombocytopenia due to other clinical conditions had increased levels of H-PF4 antibodies (data not shown). Eighteen patients who had negative platelet aggregometry test results showed antibodies to H-PF4 (Fig 1). Fifteen patients (8 men and 7 women) with a positive platelet aggregometry test result did not show antibodies to H-PF4 (Fig 1) and were studied further, as described below.

Assay of antibodies to IL-8 and/or NAP-2. The individual results of the ELISAs for the measurement of antibodies to IL-8 and to NAP-2 are shown in Fig 2. In the normal group, the mean absorbance value was 0.16 ± 0.07 for IL-8 and 0.18 ± 0.06 for NAP-2. In the groups of patients with thrombocytopenia from causes other than HAT or with autoimmune diseases, the mean value was 0.23 ± 0.11 (IL-8) and 0.21 ± 0.13 (NAP-2). The upper limit of the normal range corresponding to the absence of antibodies was thus defined as the global mean value + 3 SD: (0.21) + 3 (0.09), ie, 0.48 for IL-8; (0.20) + 3 (0.10), ie, 0.50 for NAP-2. These values did not differ significantly from those obtained using control plates without antigen. Therefore, a positive titer for the ELISA of these chemokines was defined as A492 > 0.5. In the group of 35 patients treated with heparin for more than 10 days but without HAT, antibodies to IL-8 were observed in 2 patients and to NAP-2 in 1 patient (Fig 2). Among the 72 HAT patients with positive H-PF4 antibodies who were tested, none of them had detectable antibodies to either IL-8 or NAP-2 (A492 < 0.5; Fig 2). In contrast, of the 15 HAT patients with no antibodies to H-PF4, 6 had increased anti–IL-8 antibodies and 3 had anti–NAP-2 antibodies (A492 > 0.5; Fig 2).

The main characteristics of HAT patients according to the type of antibodies detected (anti–H-PF4, NAP-2, or IL-8) are shown in Table 1. The incidence of thrombosis is higher in the group of patients with H-PF4 antibodies (55%) than those with H-PF4 antibodies (25%), although the difference is not statistically significant. Thrombocytopenia occurred later in the group of patients with NAP-2 antibodies as compared with those with H-PF4 and IL-8 antibodies. Table 2 shows further characteristics of the 9 patients with antibodies to IL-8 or NAP-2. All patients had a clinical context of inflammation and hemostatic activation, associated with either cardiac surgery, bone fracture, infection, venous thrombosis, or malignancy.
In all cases, the antibodies reacted directly with purified IL-8 or NAP-2. Heparin failed to increase the binding of antibodies at any concentration and showed a slight inhibitory effect in some cases (data not shown). Results were similar regardless of whether native or r-NAP-2 or r-IL-8 was used and whatever the source of recombinant protein, prepared by us or commercial (data not shown). The binding was specific, because no reactivity was observed with control plates processed in the same way but prepared without any specific antigen or coated with H-PF4. All 9 patients were monospecific and presented only antibodies either to IL-8 or to NAP-2. Isotyping showed the presence of IgG alone in 5 cases, IgM alone in 2 cases, and both IgM and IgG isotypes in 2 cases (Table 2). In the 9 patients, antibodies to IL-8 or to NAP-2 were still present 3 to 10 days after the withdrawal of heparin.

Four of the 9 patients with antibodies to NAP-2 or IL-8 were more thoroughly explored (patients no. 1 through 4; Table 2 and Fig 3). In all 4 patients, there was no heparin enhancing effect on antibody binding. Among the 2 patients with NAP-2 antibodies tested further, only patient no. 3 also reacted with NAP-2 precursors (β-TG, CTAP-III, and PBP; Fig 3). Neither of the two IL-8–positive patients that were tested reacted with either NAP-2 or NAP-2 precursors.

**DISCUSSION**

Our studies represent the first report of autoantibodies to NAP-2. Whereas autoantibodies to IL-8 have been previously reported at low concentrations in healthy individuals and at higher concentrations in patients with underlying inflammatory states such as malignancy, infections, major surgery, and autoimmune disease, ours is the first documentation of the association of these autoantibodies to clinical HAT. In contrast to previous observations, we did not detect antibodies to IL-8 or to NAP-2 in normal subjects or patients with autoimmune disease when using our ELISA techniques. We did detect low levels of antibodies to IL-8 and NAP-2 in a few patients without HAT who had been treated with heparin. Furthermore, in our studies of HAT, we consistently found that the patients with anti–H-PF4, anti–NAP-2, or anti–IL-8 antibodies do not have antibodies that cross-react with each other. Thus, none of...
the 72 patients with antibodies to H-PF4 had antibodies to
NAP-2 or IL-8.

Our data differ from those previously published on the
appearance of IL-8 antibodies at low levels in normal con-
trols and at higher levels in patients with underlying in-
flammatory conditions. The technical approach used by
Sylvester et al was aimed to detect very low levels of
anti–IL-8 autoantibodies, free or complexed with IL-8. Our
analysis only considered free autoantibody levels that largely
exceeded the concentrations reported in the healthy popu-
lation. We thus defined the positive range as A492 value above
the mean of control groups + 3 SD. Therefore, low antibody
levels detected below this cut-off were considered as not
significant. An alternative explanation is that, in the previous
studies, exposure to heparin may have occurred in these
patients, but its role in the pathogenesis was overlooked.

The role of heparin in the pathophysiology of this disease
is strongly supported by the finding of positive platelet ag-
eggregometry tests in these patients only in the presence of
heparin. However, the biological role of heparin in the in-
duction of the NAP-2 and IL-8 antibodies is unclear be-
cause the binding of antibodies to NAP-2 or IL-8 in our
ELISA studies did not depend on the addition of heparin. It
is possible that in vivo binding of the chemokine to the
antibody depends on heparin, but that, in the ELISA system,
no heparin is necessary. Under this scenario, the pathophys-
iology of HAT secondary to NAP-2 or IL-8 would be similar
to that due to PF4.

Alternatively, the lack of heparin dependency in our assay
system may indicate that the antibodies are not directed against
heparin-chemokine, but rather to the chemokine it-
self. In this case, the antibodies developed independently of
heparin therapy. As mentioned above, previous studies of
patients with inflammatory states have shown the presence
of circulating antibodies to IL-8 in many patients, with the
level of antibody correlating with that of IL-8. It has
been speculated that the antibody-bound IL-8 acts either as
a reserve of chemokine for high-affinity receptors or for
neutralizing the chemokines released in blood circulation.

Under these circumstances, heparin accentuates a pre-ex-
isting situation, perhaps by allowing greater mobilization of
chemokines and presenting higher concentrations to target
cells such as circulating platelets, neutrophils, and endothe-

tial cells. This complication is further enhanced by preex-
isting clinical states associated with the production of high
concentrations of chemokines.

Indeed, it has been shown that chemokines have a greater
affinity for heparin than for glycosaminoglycans. Therefore,
the infusion of heparin may induce the release of IL-
8 and NAP-2 into blood circulation and may favor the forma-
tion of heparin-chemokines complexes. It is usually accepted
that autoantibodies impair chemokines’ activity by pre-
venting their binding to cell receptors. Heparin, which
binds to platelets, leukocytes, and endothelial cells in an
upregulated manner upon cell activation, may carry chemo-
kines and target immune-complexes onto these cells. Auto-
antibodies to IL-8 and NAP-2 may then activate cells in
a similar way to H-PF4 autoantibodies, as discussed in the
introduction. This mechanism may be enhanced by cell-cell
interactions at the site of inflammation or haemostatic activa-
tion.

In conclusion, HAT is associated with the presence of
antibodies to chemokines, most of them being directed to-
wards H-PF4 complexes. In this study, we show the presence
of anti–NAP-2 and anti–IL-8 antibodies in 9 of 15 patients
with HAT showing no detectable anti–H-PF4 antibodies,
accounting for more than 10% of all patients diagnosed with
HAT. We speculate that the resulting thrombocytopenia and
thrombosis may be due to the same mechanism as in H-PF4
HAT or be due to an alternative mechanism in which heparin
mobilizes IL-8 and NAP-2 already present in these patients.
According to this second hypothesis, the patients must al-
ready have an underlying inflammatory state leading to
higher baseline chemokine levels. Finally, we speculate that
the lack of detectable antibodies in 6 patients with HAT
suggests that antibodies to other chemokines or other heparin
binding proteins might be present in these cases.

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REFERENCES

1. Weismann RI, Tobin RW: Arterial embolism occurring during
2. Gollub S, Ulin AW: Heparin-induced thrombocytopenia in
16:173, 1986
89:431, 1995
5. Amiral J, Bridey F, Dreyfus M, Vissac AM, Fressinaud E,
Wolf M, Meyer D: Platelet factor 4 complexed to heparin is the

target for antibodies generated in heparin-induced thrombocytopenia.
Thromb Haemost 68:95, 1992
chemokines of the β-thromboglobulin/interleukin 8 family. Platelets
3:295, 1992
7. Greinacher A, Potsch B, Amiral J, Dammel V, Eichner A,
Mueller-Eckhardt C: Heparin-associated thrombocytopenia: Isola-
tion of the antibody and characterization of a multimolecular PF4-
heparin complex as the major antigen. Thromb Haemost 71:247,
1994
8. Kelton JG, Smith JW, Warkentin TE, Hayward CPM, De-
nomme GA, Horsewood P: Immunoglobulin G from patients with
heparin-induced thrombocytopenia binds to a complex of heparin
patients with heparin-induced thrombocytopenia/thrombosis are spe-
cific for platelet factor 4 complexes with heparin or bound to endo-

10. Amiral J, Bridey F, Wolf M, Boayer-Neumann C, Fressinaud E,
Vissac AM, Peynaud-Debayle E, Dreyfus M, Meyer D: Antibod-
ies to macromolecular platelet factor 4-heparin complexes in hepa-in-induced thrombocytopenia: A study of 44 cases. Thromb
Haemost 73:21, 1995
38. Taub DD, Oppenheim JJ: Chemokines, inflammation and the immune system. Therapeutic Immunol 1:229, 1994
40. Tanaka Y, Adams DH, Shaw S: Proteoglycans on endothelial cells present adhesion-including cytokines to leukocytes. Immunol Today 14:111, 1993
Presence of autoantibodies to interleukin-8 or neutrophil-activating peptide-2 in patients with heparin-associated thrombocytopenia

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