

Drug eruptions and isotypic antibody responses to streptokinase after infusions of anisoylated plasminogen-streptokinase complex (APSAC, anistreplase)

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Background: Anisoylated plasminogen-streptokinase complex (APSAC, anistreplase) is a thrombolytic agent (131 kd) used for treatment of myocardial infarction. Like its principal antigenic determinant, streptokinase, APSAC has been reported to cause a variety of allergic reactions.

Objective: This study was intended to determine any association between isotypic antibody responses to streptokinase and observed allergic reactions to APSAC.

Methods: We measured sequential IgM, IgG, IgA, and IgE antistreptokinase serum levels in 21 patients who received APSAC or tissue-type plasminogen activator in a prospective, double-blind study.

Results: Of 11 patients who received APSAC, four had maculopapular rashes and one had urticaria; those with maculopapular rashes had significantly higher rises in serum IgM, IgG, IgA, and IgE antistreptokinase levels. We could not, however, define a temporal relationship between rises in antistreptokinase levels of a particular isotype and the onset of maculopapular rashes. The patient who had urticaria had no antistreptokinase responses but had also received several other potentially causal drugs. None of 10 patients who received tissue-type plasminogen activator had allergic reactions or significant rises in serum antistreptokinase levels.

Conclusion: The more vigorous panisotypic antistreptokinase responses observed in patients who received APSAC and had maculopapular rashes may reflect generalized immune system activation that included other immune responses (such as cell-mediated hypersensitivity) that were responsible for these reactions. (*J ALLERGY CLIN IMMUNOL* 1995;95:1020-28.)

Key words: Drug eruptions, antibody formation, streptokinase, tissue-type plasminogen activator, myocardial infarction

Anisoylated plasminogen-streptokinase activator complex (APSAC, anistreplase), a complex (131 kd) of human lys plasminogen and streptokinase from group C β -hemolytic streptococci, is used as a thrombolytic agent in acute treatment of

Abbreviations used

APSAC: Anisoylated plasminogen-streptokinase complex

TPA: Tissue-type plasminogen activator

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myocardial infarction.¹⁻⁶ APSAC may be administered by intravenous bolus infusion over a 3- to 5-minute period, rather than by more prolonged infusion over several hours as required for other thrombolytic agents such as streptokinase, urokinase, and tissue-type plasminogen activator (TPA).

Both APSAC and its component moiety and major antigenic determinant, streptokinase (47 kd), have been reported to cause a variety of

allergic reactions. These include anaphylaxis (associated with IgE to streptokinase), serum sickness and vasculitis (associated with IgG or IgA to streptokinase), and late nonpurpuric rashes.⁷⁻²⁰ Some but not all studies have reported that high circulating levels of antibodies to streptokinase, particularly of the IgG class, are associated with resistance to thrombolysis by streptokinase and APSAC.²¹⁻²⁸

The pathogenesis of maculopapular drug rashes is poorly understood, although several immunologic responses have been associated with rashes from different drugs. These include antibody responses of IgM and other antibody isotypes, immune complexes, lymphocytic skin infiltrates, and altered T-cell immunity.²⁹⁻³⁹ Studies of the immune responses to many low-molecular weight drugs (e.g., <1 kd), however, can be hampered by the need to assay for immune responses to multiple, chemically reactive drug metabolites that must be formed and then covalently bound to proteins to act as haptens recognized by the immune system.⁴⁰ In contrast, immune responses to high-molecular weight therapeutic agents such as proteins, specifically streptokinase and APSAC, are generally directed against antigenic determinants of the parent drug. Elevated levels of antibodies directed against the parent drug or its subunits correlate well with clinical disease that is mediated by anti-drug antibodies of a particular antibody isotype, such as IgE antibody, associated with anaphylaxis.^{11, 14, 15, 41} Consequently, studies of antibody responses to high-molecular weight proteins such as APSAC would be likely to detect antibody-mediated mechanisms if they are relevant to hypersensitivity reactions including maculopapular rashes from the agent and would give insight into any role that antibodies might play in the pathogenesis of maculopapular skin eruptions arising from drugs generally.

We prospectively obtained serum samples from patients receiving APSAC or TPA for thrombolytic treatment of myocardial infarction. We then measured sequential rises in serum levels of IgM, IgG, IgA, and IgE antibodies to streptokinase in patients after APSAC infusion and compared the degree and time course of rises in isotypic antibody titers with the development of late cutaneous drug eruptions.

METHODS

Patients

Twenty-one patients were entered prospectively into a double-blind study in which patients received either APSAC (Eminase) TPA (Alteplase) as a thrombolytic

agent for treatment of acute myocardial infarction. Patients were excluded from the study if they had received streptokinase therapy within the previous 6 months or had ever had a severe allergic reaction to streptokinase. All patients in the study provided informed consent. Patients received intravenously either (1) 30 units of APSAC (approximately 30 mg containing 1,080,000 IU streptokinase component)⁴ over 2 to 5 minutes and a placebo control for TPA over 3 hours or (2) TPA (total dose 100 mg for patients ≥ 65 kg or 1.25 mg/kg for patients <65 kg) for 3 hours and a placebo control for APSAC over 2 to 5 minutes. As part of study protocol, patients also received porcine heparin and aspirin. Other medications were administered as required. At the completion of the study, the study records were opened to reveal which patients had received APSAC and which TPA. Eleven of the 21 patients had been randomly assigned to receive APSAC.

Serum samples

Serum samples were obtained immediately before infusion (baseline) and at selected intervals after infusion, generally 1 day, 3 days, 10 days (at discharge if discharged before 10 days), and 1 month after infusion.

ELISAs

Serum levels of IgM, IgG, IgA, and IgE to streptokinase were determined in triplicate by ELISA with minor modifications of protocols previously published.^{11, 15, 42, 43} The following agents were added in sequence to Maxi-sorp-Immunoplate microtiter plates (Nunc, Roskilde, Denmark) with appropriate washes and incubation periods: streptokinase, dilutions of patient serum samples, either rabbit anti-human IgM, IgG, or IgA (Sigma Chemical Company, St. Louis, Mo.) followed by alkaline phosphatase-conjugated goat anti-rabbit gamma globulin (Sigma) or goat anti-human IgE followed by alkaline phosphatase-conjugated rabbit anti-goat gamma globulin (Sigma), and *p*-nitrophenyl phosphate (Sigma). The optical density of each well was then read at 405 nm by means of an automated ELISA reader.

Statistical analysis

Anti-streptokinase levels of patients receiving APSAC who did and did not have skin eruptions were compared with those of patients who received TPA by paired *t* test (for preinfusion levels) and by ANCOVA (for postinfusion levels) by means of SPSS for Windows software (SPSS Inc., Chicago, Ill.). In an individual patient, a significant rise in an isotypic antibody level during the postinfusion period was defined as a greater than two-fold increase in the optical density of the ELISA result.

RESULTS

Patients and reactions

Four of the 11 patients receiving APSAC had maculopapular skin reactions from 5 to 9 days after APSAC infusion, and one patient had urti-

TABLE I. Patients with skin eruptions after APSAC

No.	Age (yr)	Pertinent history	Reactions	Onset	Other new medications	Treatment regimen
1	59	No history of allergy	Generalized maculopapular rash	8 days after APSAC	Nitrates, diltiazem, metoprolol, furosemide, warfarin, benzodiazepines	Prednisone given for 2 days after rash development
2	48	No history of allergy	Generalized maculopapular rash	5 days after APSAC	Heparin, nitrates, metoprolol, morphine, lidocaine, phenothiazines, benzodiazepines	Prednisone given for 3 days after rash development
3	51	Solar urticaria	Fever to 103.9° F, generalized maculopapular rash	5 days after APSAC	Heparin, nitrates, diltiazem, metoprolol, phenothiazines, benzodiazepines, indomethacin, furosemide	Prednisone given for 1 week after rash development
4	71	Penicillin rash	Macular rash on upper extremities	9 days after APSAC	Heparin, nitrates, metoprolol, and famotidine	
5	53	No history of urticaria or recent viral syndrome	Mild urticaria on face and neck	8 days after APSAC	Heparin, nitrates, metoprolol, lidocaine (also received aspirin)	Treated with diphenhydramine as needed

All patients were white; all but patient 4 were male.

caria 8 days after APSAC infusion and after use of other drugs (Table I). Except for elevated sedimentation rates and the elevations in serum aspartate aminotransferase, creatine kinase, and lactate dehydrogenase that would be expected after myocardial infarction, no significant abnormalities of complete blood cell count, blood chemistry studies, or urinalysis were observed. No patient who received TPA had skin eruptions.

ELISA results

Patients receiving APSAC. Serum levels of IgM, IgG, IgA, and IgE antibodies to streptokinase before (day 1, baseline) and after APSAC infusion are reported for individual patients in Figs. 1 to 4. Of the four patients who had maculopapular rashes after receiving APSAC, all had significant rises in antistreptokinase IgM, IgG, and IgA antibodies, and three also had significant rises in antistreptokinase IgE. Of the six patients who did not have skin eruptions, four had significant rises in antistreptokinase IgG and IgA and three also had significant rises in antistreptokinase IgM. The one patient who had urticaria after administration of APSAC and other drugs did not have significant rises in antistreptokinase antibodies of any isotype.

Preinfusion antistreptokinase antibody levels were not significantly different between those who did and did not have maculopapular skin eruptions (Table II). During the 10-day period after APSAC

infusion when skin eruptions began, patients who had maculopapular rashes had serum levels of antistreptokinase IgM, IgG, IgA, and IgE that were significantly higher than those of other patients receiving APSAC. Maculopapular rashes sometimes developed before a significant rise in antibody levels, however, and levels of antibodies to streptokinase that were present in patients with rashes at the onset of their maculopapular rashes were not higher than antibody levels that eventually developed in some patients who did not have rashes. During a follow-up of 30 to 45 days after APSAC administration, some patients who received APSAC and did not have skin rashes had higher antibody levels than during the first 10 days after APSAC use, in contrast to patients who received APSAC and had maculopapular rashes, who had peak antistreptokinase antibody levels during the course of their skin rashes in the first 10 days after APSAC administration.

Patients receiving TPA. Preinfusion antistreptokinase antibody levels did not differ significantly between patients who received APSAC and those who received TPA. No patients who received TPA had significant rises in antistreptokinase antibody levels of any immunoglobulin class.

DISCUSSION

Patients who had maculopapular rashes after APSAC administration also had significantly

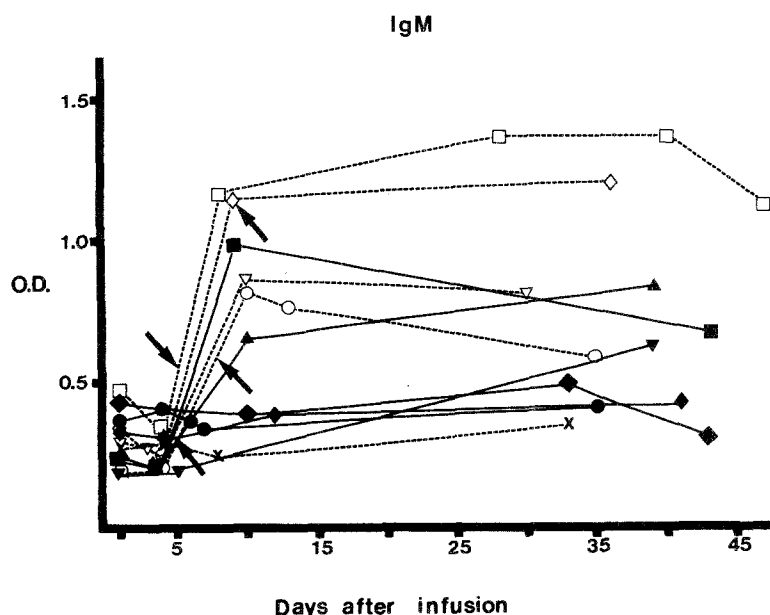


FIG. 1. Serum level of IgM antibody to streptokinase in patients who received APSAC, as assayed by ELISA. Serum dilution of 10^{-3} . Optical density (OD) is plotted against time after APSAC infusion (infusion on day 1). Data are plotted with *open symbols* for patients with maculopapular rashes, *closed symbols* for nonreacting patients, and *X* for a patient who had urticaria. Arrows indicate onset of maculopapular rash (see Table I).

higher rises in serum antistreptokinase levels of all four antibody classes (IgM, IgG, IgA, and IgE) than did patients who received APSAC but did not have maculopapular rashes. We could not, however, clearly define a temporal relationship between the development of rises in antistreptokinase levels of a particular immunoglobulin class and the onset of skin eruptions. In several cases, antistreptokinase antibody levels rose only several days after the development of maculopapular rashes. In other cases, antistreptokinase antibody levels had become elevated above baseline levels at the onset of maculopapular rashes, but levels at that time were not higher than those that eventually developed in other patients who did not have rashes. These observations, although made in relatively few patients, would seem to indicate that antibodies to streptokinase were not responsible for the maculopapular rashes observed in the patients who received APSAC. However, the more vigorous antistreptokinase antibody responses of multiple immunoglobulin classes that developed in the patients with maculopapular rashes demonstrate that these patients had greater, generalized, antigen-specific activation of their immune systems. We propose that as part of this greater generalized immune system activation, patients with maculopapular rashes had non-antibody-me-

diated, antigen-specific immune responses that were not studied (such as cell-mediated mechanisms) that were responsible for the skin rashes, because antibody-mediated responses to streptokinase do not appear to explain the development of maculopapular rashes in our patients. Although reports in the literature are conflicting, a role for cell-mediated immunity in the pathogenesis of nonurticarial drug rashes has been suggested by the association of several immunologic abnormalities with such rashes. These include lymphocytic skin infiltrates, increased numbers of Langerhans cells in lesional skin, in vitro proliferation of lymphocytes in response to drugs (correlating best with rashes from antiepileptic medications, correlating variably to poorly with sulfonamide rashes), T-cell cytokine production in response to causative drugs, and the culturing of drug-reactive lymphocytes from peripheral blood.³⁴⁻³⁹

The development of antistreptokinase antibody responses in patients who received APSAC and did not have allergic reactions is not unexpected. Studies of antibody responses after administration of APSAC, streptokinase, other high-molecular weight proteins such as insulin and heterologous antibodies, and low-molecular weight drugs such as antibiotics have shown that many more patients have immune responses, particularly non-IgE iso-

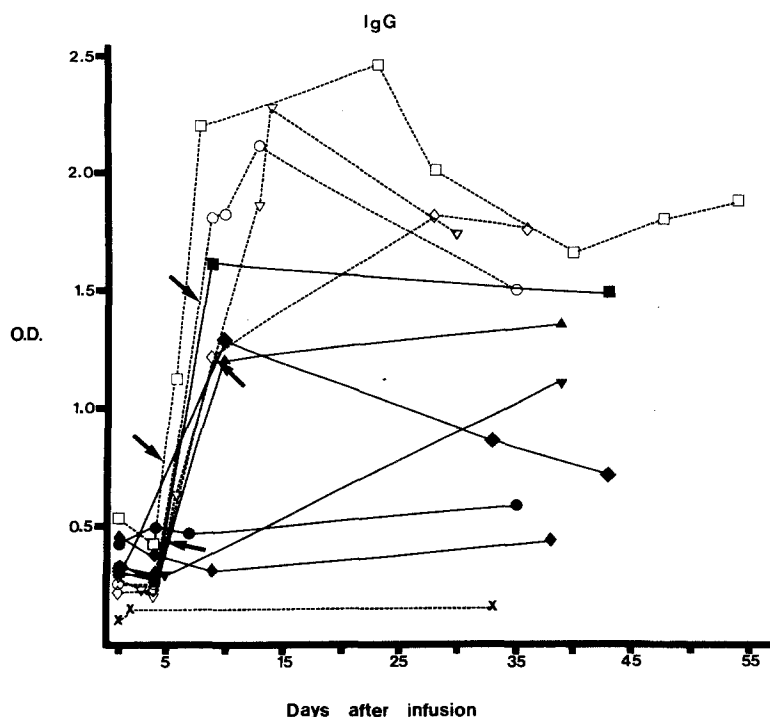


FIG. 2. Serum level of IgG antibody to streptokinase in patients who received APSAC, as assayed by ELISA. Serum dilution of 10^{-5} . Optical density (OD) is plotted against time after APSAC infusion (infusion on day 1). Data are plotted with *open symbols* for patients with maculopapular rashes, *closed symbols* for nonreacting patients, and *X* for a patient who had urticaria. Arrows indicate onset of maculopapular rash (see Table I).

type antibody responses, than have clinical allergic reactions mediated by such immune responses.^{23, 40, 41, 44, 45} In the evaluation of drug allergy, this response has limited the clinical usefulness of assays available for detection of drug-specific immune responses and emphasizes the importance of recognizing that immune responses are not equivalent to immunologically mediated clinical disease. Differences between patients in target organ susceptibility to immune responses may be a factor in explaining why some patients who have immune responses also have clinical allergic disease whereas others do not. For this reason, we cannot completely exclude a role for antibodies to streptokinase in the development of maculopapular rashes from APSAC in patients who did have antibodies present at the onset of their rashes, even though such levels were not higher than those that eventually developed in patients who did not have maculopapular rashes.

None of the patients who received TPA had significant rises in antistreptokinase antibody levels, indicating that patients who received APSAC had significant rises in antistreptokinase levels because of their exposure to the antigenic strep-

tokinase moiety in APSAC, not because of exposure to streptococcal organisms in the hospital or their morbid state.

Varying levels of antistreptokinase antibodies are present in a large proportion of the general population, probably as a result of nearly ubiquitous exposure to streptococci and common infections with these organisms.⁴⁶ Consequently, the more vigorous antistreptokinase antibody responses in the patients who received APSAC and had maculopapular rashes probably represent anamnestic rather than primary responses to streptokinase in APSAC. Because the baseline levels of antistreptokinase antibodies of all four isotypes were similar in patients who received APSAC with and without maculopapular rashes, however, there is no evidence that preexisting exposure or immunologic sensitization to streptokinase was higher in the patients with maculopapular rashes, although this does not preclude the possibility that these patients had higher preexisting, nonhumoral (cell-mediated) sensitivity to streptokinase.

The relatively high incidence of maculopapular rashes in our study group contrasts with a reported incidence of 1% to 2.8% in large studies.^{2, 4, 24} It

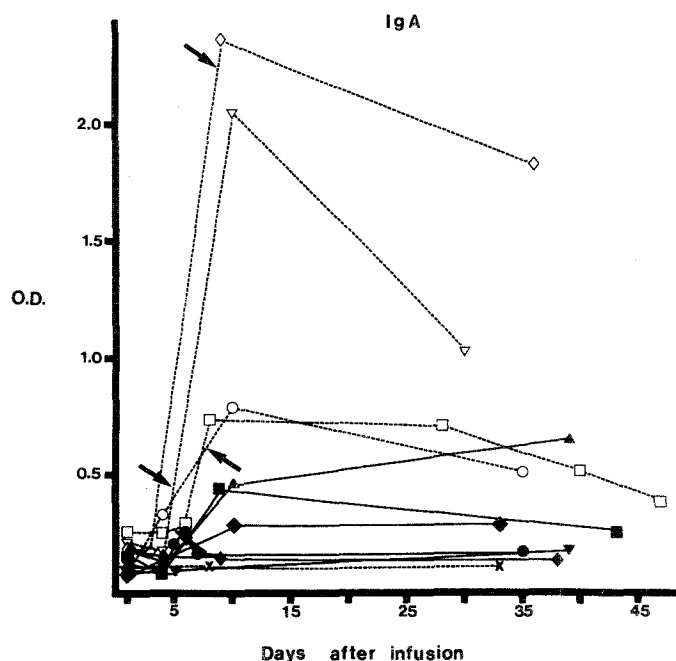


FIG. 3. Serum level of IgA antibody to streptokinase in patients who received APSAC, as assayed by ELISA. Serum dilution of 10^{-4} Optical density (OD) is plotted against time after APSAC infusion (infusion on day 1). Data are plotted with *open symbols* for patients with maculopapular rashes, *closed symbols* for nonreacting patients, and *X* for a patient who had urticaria. Arrows indicate onset of maculopapular rash (see Table I).

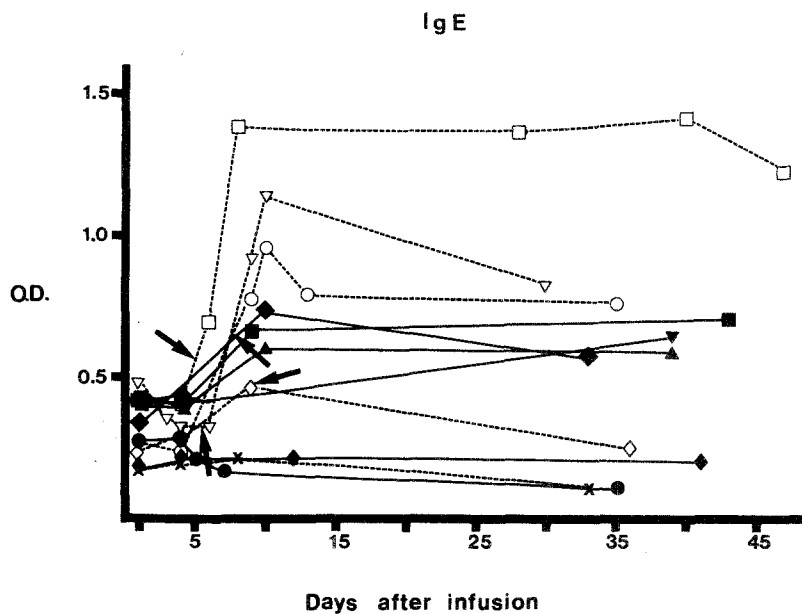


FIG. 4. Serum level of IgE antibody to streptokinase in patients who received APSAC, as assayed by ELISA. Serum dilution of 10^{-3} Optical density (OD) is plotted against time after APSAC infusion (infusion on day 1). Data are plotted with *open symbols* for patients with maculopapular rashes, *closed symbols* for nonreacting patients, and *X* for a patient who had urticaria. Arrows indicate onset of maculopapular rash (see Table I).

TABLE II. Serum levels of IgM, IgG, IgA, and IgE antibodies to streptokinase by ELISA at baseline and after infusion of APSAC or TPA

	APSAC, no rash (<i>n</i> = 6)	APSAC, maculopapular rash (<i>n</i> = 4)	APSAC, urticaria (<i>n</i> = 1)	TPA (<i>n</i> = 10)
IgM				
Baseline	0.30 ± 0.10	0.32 ± 0.12	0.27	0.38 ± 0.06
Peak, within 10 days	0.45 ± 0.26	1.00 ± 0.18 (<i>p</i> < 0.012)*	0.29	0.43 ± 0.08
Peak, entire follow-up	0.60 ± 0.22	1.06 ± 0.27 (<i>p</i> < 0.026)*	0.35	0.44 ± 0.08
IgG				
Baseline	0.36 ± 0.08	0.33 ± 0.15	0.14	0.32 ± 0.05
Peak, within 10 days	0.87 ± 0.56	1.88 ± 0.48 (<i>p</i> < 0.030)*	0.14	0.33 ± 0.05
Peak, entire follow-up	1.04 ± 0.51	2.18 ± 0.28 (<i>p</i> < 0.007)*	0.17	0.37 ± 0.13
IgA				
Baseline	0.13 ± 0.04	0.19 ± 0.07	0.14	0.15 ± 0.06
Peak, within 10 days	0.27 ± 0.16	1.59 ± 0.75 (<i>p</i> < 0.010)*	0.14	0.17 ± 0.04
Peak, entire follow-up	0.32 ± 0.19	1.59 ± 0.75 (<i>p</i> < 0.013)*	0.14	0.17 ± 0.04
IgE				
Baseline	0.33 ± 0.10	0.29 ± 0.20	0.18	0.30 ± 0.08
Peak, within 10 days	0.46 ± 0.19	0.99 ± 0.39 (<i>p</i> < 0.019)*	0.22	0.35 ± 0.05
Peak, entire follow-up	0.51 ± 0.20	1.00 ± 0.40 (<i>p</i> < 0.032)*	0.22	0.37 ± 0.09

Values are optical density readings, mean ± SD.

*Significant difference between APSAC, no rash and APSAC, maculopapular rash groups.

may be that the incidence of skin eruptions in our relatively small sample of patients may not be representative of the true incidence of skin eruptions caused by APSAC, although it also may be that rashes from APSAC are underreported elsewhere, particularly if mild. We cannot exclude the possibility that maculopapular rashes in some of our patients receiving APSAC may have been caused by concomitantly administered, newly introduced drugs such as calcium-channel blockers or β -blockers, although the frequency of allergic reactions to these drugs is low.^{47, 48} We believe that APSAC is strongly implicated as the etiologic agent, however, because no rashes developed in the patients who received TPA, even though the patients in the TPA and APSAC groups received similar nonthrombolytic drugs.

We believe that late-onset urticaria observed in one of the patients who received APSAC and had no history of idiopathic urticaria may have been caused by one of several concomitantly administered medications such as aspirin and not by APSAC. Because immunologically mediated urticaria from drugs is generally associated with IgE or IgG antibodies to the causative drug or its antigenic determinants, the lack of significant anti-streptokinase IgE or IgG antibody production in this patient with urticaria argues that APSAC was not responsible for the urticaria.

Because APSAC in aqueous-based solutions dis-

sociates into streptokinase, plasminogen, and anisic acid, which precludes the development of in vitro assays for antibodies to whole-complex APSAC,⁴ we did not measure serum levels of antibodies to APSAC itself. It could be argued that because we assayed for antibodies to streptokinase and not to APSAC we may have missed detection of antibody responses to potential antigenic determinants unique to APSAC or its breakdown products, and that these responses may have been responsible for the maculopapular rashes or urticaria observed in our patients. We believe this is unlikely for several reasons. First, lys plasminogen, the major moiety in APSAC other than streptokinase, is derived from human plasma and is unlikely to be highly antigenic. Second, it is unlikely that anisic acid or anisoyl determinants would be significant immunogens because anisic acid (4-methoxybenzoic acid) is a low-molecular weight compound (152 daltons) that is cleared after conversion to its glycine and glucuronide conjugates and is relatively unreactive with proteins in vivo, anisoyl-albumin conjugates (chemically prepared in vitro) do not induce conjugate-specific antibody responses in animal models, and the anisoyl group is present on APSAC only transiently in vivo, and its binding site to plasminogen is in the interior of the APSAC tertiary structure and thus probably inaccessible to immune surveillance.^{4, 49, 50} Third, it is unlikely that significant neoantigens are formed,

not only because of the preceding arguments but also because the dissociation of the anisoyle group from its binding site on plasminogen does not lead to structural alteration of plasminogen and lys plasminogen and streptokinase are not covalently linked in APSAC.⁴ Fourth, previous studies have shown that antistreptokinase antibody responses and the frequency of allergic reactions to APSAC and streptokinase are similar, indicating that the streptokinase moiety in APSAC is the significant antigenic determinant in APSAC.^{4, 23, 24}

Although our data in this study indicate that antibodies to streptokinase are probably not responsible for maculopapular drug rashes from APSAC, there are data demonstrating a role for IgE to streptokinase in anaphylaxis, and IgG or IgA to streptokinase in serum sickness and vasculitis from streptokinase and APSAC.^{7, 11, 14, 15, 19, 20} It has been previously reported that antistreptokinase IgG serum levels can fall immediately after infusion of APSAC or streptokinase because of sequestration as the antigen-antibody complex, return to near baseline levels within 5 days, peak at 14 days after infusion, and then return to baseline levels in most patients after 6 to 12 months.^{23, 25} This pattern has led to recommendations that streptokinase or APSAC not be re-administered during the period from 5 days until 6 to 12 months after an earlier dose of streptokinase because of the likelihood of resistance to thrombolysis as a result of antistreptokinase neutralizing antibody or the possibility of increased risk of antibody-mediated allergic reactions such as anaphylaxis and serum sickness. In this study, which to our knowledge is the first to examine sequential antistreptokinase serum levels of the four major antibody isotypes after infusion of either APSAC or streptokinase, we found that antistreptokinase serum levels of all four isotypes began to rise above baseline levels 5 days after APSAC administration, peaked after 10 to 15 days in most patients, and in some patients persisted at elevated levels throughout the approximately 30 day follow-up period. These findings underscore the concern that there is increased risk for antibody-mediated allergic reactions, including IgE-mediated immediate-type reactions and IgG- or IgA-mediated serum sickness-like reactions, if APSAC or streptokinase is readministered after recent use of APSAC or streptokinase.

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