Staging of the Baboon Response to Group A Streptococci Administered Intramuscularly: A Descriptive Study of the Clinical Symptoms and Clinical Chemical Response Patterns

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Group A streptococcal infections, ranging from necrotizing fasciitis and myositis to toxic shock syndrome, have increased over the last 10 years. We developed the first primate model of necrotizing fasciitis and myositis. Thirteen baboons were inoculated intramuscularly with group A streptococci (GAS). Eleven animals survived for >11 days before sacrifice, and two animals died within 2 days. The site of inoculation of the survivors exhibited an intense neutrophilic influx (stage I), followed by a lymphoplasmaclastic influx (stages II and III). This was accompanied by the appearance of markers of an acute and then a chronic systemic inflammatory response. In contrast, the site of inoculation of the two nonsurvivors exhibited intravascular aggregates of neutrophils at its margin with no influx of neutrophils and with extensive bacterial colonization. We conclude that GAS inoculation induces a local and systemic acute neutrophilia followed by a chronic lymphoplasmaclastic response; failure, initially, of neutrophilic influx into the site of inoculation predisposes to systemic GAS sepsis and death; and this three-stage primate model approximates the human disease.

The incidence of group A streptococcal (GAS) infections has increased over the last 10 years [1]. This disorder presents in various forms, which range from self-limiting to fatal. The most common presentations range from pharyngitis [2], necrotizing fasciitis [3–5], and myositis [1, 6, 7] to streptococcal toxic shock syndrome (TSS) [8–17]. These entities, in turn, can run a course leading to bacteremia, shock, acute respiratory distress syndrome, renal failure, and aggressive soft-tissue destruction [1]. The overall mortality of patients presenting with necrotizing fasciitis and myositis is 30% [1, 2] and 80% [1, 6, 18–22], respectively. The portal of entry often is the skin or mucous membranes, although a definite portal of entry cannot be ascertained in 45% of cases [1, 23]. Of those patients diagnosed as having streptococcal TSS, most present with fever and tachycardia (80%), blood pressure below 110 mm Hg (55%), confusion (55%), and profound hypothermia and shock (10%) [1]. The development of a primate model of GAS infection is based on the fact that 80% of patients with streptococcal TSS developed evidence of soft-tissue infection in which localized swelling and erythema were the most common findings at the time of admission [22].

The first objective of this study was to correlate the systemic inflammatory and hemostatic events with the three stages of the development of the soft-tissue lesions (fasciitis and myositis); the second objective was to assess how closely this primate model mimics streptococcal TSS seen in humans; and the third objective was to compare the systemic and local tissue responses at the site of injection of GAS in the nonsurvivors with those of the survivors.

Materials and Methods

Bacteria. GAS strain DLS 85 W was an isolate from blood of a patient with clinical features that met all of the criteria of streptococcal TSS, including bacteremia, intractable shock, renal failure, acute respiratory distress syndrome, necrotizing fasciitis, and death. The strain was M type 3 (typed by Edward Kaplan, WHO Streptococcal Reference Laboratory, University of Minnesota) that produced pyrogenic exotoxin A (determined by Patrick Schlievert, University of Minnesota), pyrogenic streptococcal superantigen (determined by Kristen Reda, Baylor University, Houston), and mitogenic factor (determined by Stig Holm, Umeå, Sweden). The strain was found to belong to Lancefield group A on the basis of carbohydrate antigens, was β-hemolytic on sheep blood agar, and was sensitive to bacitracin, penicillin, and ceftriaxone (ceftriaxone MIC, 0.012 μg/mL).
Bacterial culture and inoculum preparation. Streptococci were grown in Todd-Hewitt broth (Difco, Detroit). All cultures were incubated at 37°C in 5% CO₂. After primary isolation of the organism, 50 mL of Todd-Hewitt broth was inoculated and incubated for 18 hours at 37°C in 5% CO₂. This culture was inoculated into 500 mL of fresh broth and incubated for another 4 h. This log-phase culture was centrifuged at 4000 g for 10 minutes, the supernatant was removed, and the pellet was washed once with sterile 0.9% NaCl. The pellet was suspended in 0.9% NaCl to provide 1–3 × 10¹⁰ cfu/mL. This preparation, or dilutions thereof, was used as the inoculum.

Animal preparation and procedures. The experimental methodology has been described previously [24]. Thirteen baboons (Papio cynocephalus cynocephalus) of either sex were used; all had hematocrits of >36% and were free from tuberculosis. They were divided into two groups (table 1). The initial studies were done on nine animals (group 1, animals 18–26). On the basis of these studies, a second series, which included collection of tissue samples from the site of injection of the GAS organisms, was done (group 2, animals 27–30). The baboons were fasted overnight before each experiment and given water ad libitum. Each animal was sedated with ketamine hydrochloride (14 mg/kg) at the time of im injection of GAS at 5.6 × 10⁹ to 1.1 × 10¹⁰ cfu/kg into the left thigh. The animals were returned to the cage, and vital signs were determined, or dilutions thereof, was used as the inoculum.

Survivors were sacrificed at 22 days (group 1) and at 11 days (group 2). The venous blood collected at baseline included the following samples: 1 mL anticoagulated with EDTA for WBC and platelet counts and differential and hematocrit determinations; 2 mL anticoagulated with 3.8% sodium citrate for fibrinogen determination [25] and ELISAs for cytokines and enzyme/inhibitor complexes (see below); 0.5 mL in trasylol/thrombin for measurement of fibrin degradation products [26]; 3 mL of clotted blood for measurement of blood urea nitrogen [27], creatinine [28], serum glutamic pyruvic transaminase [29], creatine phosphokinase (CPK) [30], electrolytes [31], and cortisol [32]; and 1 mL anticoagulated with potassium oxalate for lactate measurement [33]. The arterial blood collected included 0.5 mL of nonanticoagulated blood for glucose measurement [33] and 0.5 mL of whole blood for bacterial colony counts [34]. Not more than 10% of the estimated total blood volume (70 mL/kg) was withdrawn over the first 24 h.

ELISAs and RIA. The following assays were done as described previously: ELISA for thrombin/antithrombin complex [35]; RIA for plasmin/α₂-antiplasmin [36]; ELISAs for tissue plasminogen activator activity [37] and plasminogen activator-inhibitor type 1 [38]; ELISAs for endostatin [39] and C3b/c and C4b/c [40]; and ELISAs for IL-8 [41–43], TNF [43], IL-6 [44], IL-10 [45], IL-12 [45, 46], IFN-γ [46], and phospholipase A₂ [47].

Tissue biopsy at injection site, postmortem examination. Elliptical segments (3 × 1 cm) of tissue, including skin, subcutaneous, and muscle tissue, were obtained at 24 h and 7 and 11 days after inoculation from 4 of the 13 animals (animals 27–30). Tissue samples were fixed in formaldehyde and stained with hematoxylin-eosin for determination of cellular architecture and with phosphotungstic acid–hematoxylin for visualiza-
tion of fibrin. Postmortem examinations were done for all 13 animals, at either the time of death (animals 18 and 23) or the time of sacrifice. For group 1, this was at 21 days, at which time recovery was complete or nearly complete. For group 2, sacrifice was at 11 days after inoculation, at which time the induration at the site of injection was at its height. At these times, tissue specimens were removed from the lungs, kidney, liver, adrenal glands, heart, and spleen as well as from the injection site for light microscopic examination.

**Scoring of tissue histopathology and statistical analysis.** The pathological lesions of adrenal glands, kidneys, and lungs were analyzed by dividing their description into five categories: thrombosis, hemorrhage, congestion, WBC influx, and necrosis. The tissues were rated according to the severity of the histopathologic lesions. The scale ranged from 0 to 4+, with 4+ being the most severe. All microscopic sections were read by one of us (S.D.K.), who was blinded as to which group was being analyzed. The Kruskal-Wallis test, a nonparametric test, was used to determine significant differences \( P < .05 \) between groups for a given pathological lesion.

The clinical data were analyzed by analysis of variance with Duncan’s multicomparison test to determine significant differences \( P < .05 \) between groups at given times and within certain variables. Analysis of variance, repeated-measure design, with Dunnett’s multicomparison test was also used to determine significant differences \( P < .05 \) between baseline and subsequent times for a given group.

**Results**

**Clinical Staging of the Responses of Survivors to Intramuscular Injection of GAS**

*General.* Eleven of the 13 animals in groups 1 and 2 were survivors (defined as animals that survived \( \geq 11 \) days after i.m. inoculation with GAS), while the other 2 died within 63 hours. Figure 1A shows the three stages into which the responses of these survivors were divided. These stages were based on the status of the wound at the site of injection. Stage I consisted of the initial flare, erythema, and edema and ran from the time of inoculation through 2 days afterwards. Stage II consisted of induration and extension of the wound to form an obvious abscess and ran from 3 through 6 days after inoculation. Stage III consisted initially of a crisis in which the infections reached maturity, followed by suppuration and either resolution or the persistence of a chronic inflammatory response. This stage ran from 7 through 21 days after inoculation. The cellular infiltrate of the wound at the site of injection during stages I and II was dominated by neutrophilic phagocytes, whereas that of stage III was dominated by lymphoplasmacyttes.

*Stage I (0–2 days).* The development of wound erythema and edema (figure 1A) was accompanied by an initial rise in neutrophil count and a decline in lymphocyte count (figure 1B). There also was a fourfold rise in fibrinogen concentration during this stage, while the albumin concentration remained constant before falling in stage II. Among the survivors, the leukocytosis was accompanied by seven- and fivefold rises in elastase/\( \alpha_1 \)-antitrypsin complex and IL-6 concentrations, respectively, both of which peaked at 1–2 days (figure 1C). There were also four- and sixfold rises in CPK and serum glutamic pyruvic transaminase levels, respectively, both of which peaked at 2 days (figure 1D). In contrast, the markers of renal function (blood urea nitrogen and creatinine levels) did not change. There was no rise in the concentration of TNF, even in those animals (animals 27–30) from which samples were collected at 2, 4, and 6 hours, and only a limited rise in thrombin/antithrombin complex during stage I (table 2).

The histopathology at the site of injection at 24 h was characterized by an acute neutrophilic infiltration into the subcutaneous tissue, underlying fascia, and skeletal muscle (figure 2, left). This infiltration was most intense in the subcutaneous tissue and fascia. The influx of neutrophils into the musculature was characterized by accumulation along the endomysial surface of the myofibrillar bundles (figure 2, right). Marked edema of the dermis, subcutaneous tissue, and fascia also was observed. There was some necrosis of myofibrillar bundles and no evidence of accumulation and margination of neutrophils in vessels. No bacterial aggregates were observed. This histopathologic response is in contrast to that of the two nonsurvivors (animals 18 and 23), both of which had bacterial colonies within the site of injection with little, if any, neutrophilic infiltration into that site (see below).

*Stage II (3–6 days).* A persistent rise in WBC count accompanied the wound induration during stage II (figure 1B). The fibrinogen concentration reached a peak more than fourfold above baseline during day 3 of this stage (figure 3). No plasmin/antiplasmin complex or fibrin degradation products appeared in the circulation during stage II, despite rises in the concentrations of fibrinogen and the transitory and relatively limited increases in concentrations of tissue plasminogen activator (table 2, figure 3). The high concentrations of elastase/\( \alpha_1 \)-antitrypsin complex, IL-6, CPK, and serum glutamic pyruvic transaminase in stage I all returned toward normal in stage II (figures 1C, 1D), while TNF-\( \alpha \) rose transiently for the first time on day 7 of stage II (table 2). The histopathology at the site of injection at 7 days was characterized by a marked chronic/active inflammatory response, with infiltration of lymphoplasmacyttes and eosinophils as well as neutrophils, involving primarily the subcutaneous and outer skeletal muscle layers (figure 4). Note that the fascial plane between the subcutaneous tissue and muscle at this stage was markedly thickened, with a chronic/active cellular infiltrate.

*Stage III (7–21 days).* Figure 5 shows the status of the wound at the site of injection (as in figure 1A) in relation to markers of chronic inflammatory activity. These include the absolute lymphocyte and platelet counts, which gradually rose and reached a peak in the middle of stage III (10–14 days), and
the concentrations of C-reactive protein and C4b/C-reactive protein complex, which, after peaking during stage I, peaked again along with C4b/c during the middle of stage III. These were accompanied by a 50% decline in albumin concentration (figure 1B), a twofold rise in IgG concentration (not shown), and a sustained elevation of the sedimentation rate (not shown). The response of the markers shown in figures 1–5 as well as others are summarized in table 2.

The histopathology at the site of injection at 11 days was similar to that observed at 7 days, except that the cellular response, including neutrophils and lymphoplasmacytic cells, appeared to be more rather than less intense. Because all four animals in group 2 (animals 27–30) were sacrificed at 11 days, tissues removed from vital organs also were submitted for histopathologic study. The lungs and spleen showed marked neutrophilic influx (+3.25) characterized by intravascular neutrophil aggregation and margination. This was accompanied by congestion ranging from moderate (+1.5) to severe (+3.0). By contrast, the congestion (+0.5) and neutrophilic influx (+1.0) in the liver was mild. All other organs, including heart, lung, adrenals, gallbladder, pancreas, and small intestine, appeared essentially normal.

Comparison of the Major Characteristics of Streptococcal TSS in Humans and in the Baboon Model

Table 3 summarizes the similarities between the human disease and the baboon model of streptococcal TSS. The responses of all 13 of the baboons to im injection of GAS paralleled those seen in streptococcal TSS in humans. These included the appearance of the cutaneous lesions, fever, a normal or elevated WBC count with a shift to younger cells, the appearance in plasma of cytokines, a decrease in albumin and increases in fibrinogen and C-reactive protein concentrations, increases in the concentrations of markers of muscle and liver injury (CPK, serum glutamic pyruvic transaminase), and histopathologic evidence of myonecrosis and necrotizing fasciitis.

The two nonsurvivor baboons exhibited all of these markers of streptococcal TSS. In addition, these animals exhibited a fall in WBC count, hypotension, acute respiratory distress syndrome, an increase in creatinine concentration, coagulopathy, and histopathologic evidence of large concentrations of streptococci at the site of infection. The early decline instead of no
Table 2. Sequence of events during group A streptococcal (GAS) disease in baboons injected with GAS.

<table>
<thead>
<tr>
<th>Markers of phagocyte function</th>
<th>Time point (d)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>9–11</th>
<th>15–16</th>
<th>18–19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseplate forms (×10^9/m/L)</td>
<td>5.7 ± 1.1</td>
<td>3.8 ± 0.4</td>
<td><em>&lt;2.6</em></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Band forms (×10^9/m/L)</td>
<td>4.5 ± 0.5</td>
<td>2.2 ± 0.8</td>
<td>1.3 ± 0.4</td>
<td>1.1 ± 0.1</td>
<td>1.6 ± 0.9</td>
<td>1.8 ± 0.6</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.4</td>
<td>0.2 ± 0.02</td>
</tr>
<tr>
<td>Elastase (ng/mL)</td>
<td>33 ± 7</td>
<td>245 ± 39</td>
<td>200 ± 33</td>
<td>159 ± 32</td>
<td>110 ± 21</td>
<td>113 ± 27</td>
<td>119 ± 36</td>
<td>101 ± 32</td>
<td>77 ± 15</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>38.3 ± 0.2</td>
<td>39.2 ± 0.1</td>
<td>39.1 ± 0.1</td>
<td>38.7 ± 0.1</td>
<td>38.4 ± 0.3</td>
<td>38.9 ± 0.2</td>
<td>38.5 ± 0.3</td>
<td>38.0 ± 0.1</td>
<td>38.3 ± 0.2</td>
</tr>
<tr>
<td>Lactate (mEq/L)</td>
<td>5.8 ± 1.3</td>
<td>6.2 ± 1.3</td>
<td>5.0 ± 0.6</td>
<td>3.7 ± 0.3</td>
<td>3.4 ± 0.8</td>
<td>4.2 ± 0.6</td>
<td>2.4 ± 0.3</td>
<td>3.0 ± 0.6</td>
<td>2.9 ± 0.6</td>
</tr>
</tbody>
</table>

Acute inflammatory mediators and products

<table>
<thead>
<tr>
<th>Markers of phagocyte function</th>
<th>Time point (d)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>9–11</th>
<th>15–16</th>
<th>18–19</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-PA (ng/mL)</td>
<td>3.3 ± 0.6</td>
<td>7.9 ± 3.1</td>
<td>3.6 ± 1.0</td>
<td>3.4 ± 0.7</td>
<td>3.5 ± 1.0</td>
<td>4.9 ± 1.5</td>
<td>3.5 ± 0.7</td>
<td>1.6 ± 0.0</td>
<td>1.6 ± 0.0</td>
</tr>
<tr>
<td>Fibrinogen (%)</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>0.4 ± 0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>FDP (µg/dL)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>11.4 ± 1.4</td>
<td>21.6 ± 11.7</td>
<td>200 ± 6.3</td>
<td>26.7 ± 11.7</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Platelets (×10^9/m/L)</td>
<td>361 ± 21</td>
<td>311 ± 18</td>
<td>*238 ± 29</td>
<td>284 ± 23</td>
<td>335 ± 36</td>
<td>517 ± 44</td>
<td>617 ± 59</td>
<td>678 ± 37</td>
<td>571 ± 44</td>
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</table>

Chronic inflammatory mediators and products

<table>
<thead>
<tr>
<th>Markers of phagocyte function</th>
<th>Time point (d)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>7</th>
<th>9–11</th>
<th>15–16</th>
<th>18–19</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-PA (ng/mL)</td>
<td>2.4 ± 2.3</td>
<td>6.9 ± 2.1</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen (%)</td>
<td>&lt;0.4</td>
<td>0.6</td>
<td>&lt;0.2</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FDP (µg/dL)</td>
<td>&lt;10</td>
<td>15 ± 7</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets (×10^9/m/L)</td>
<td>360 ± 42</td>
<td>*290 ± 96</td>
<td>301</td>
<td></td>
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</table>

NOTE. Data are mean ± SE. Boldface indicates peak values. In survivors, blood pressure from baseline to 21 days ranged from 74 to 88 mm Hg; in nonsurvivors, blood pressure at baseline ranged from 90 to 110 mm Hg and at 2 days ranged from 40 to 57 mm Hg. CPK = creatine phosphokinase; CRP = C-reactive protein; FDP = fibrin degradation products; PAP = plasmin/α2-antiplasmin; SGOT = serum glutamic pyruvic transaminase; TAT = thrombin/antithrombin complex; T-PA = tissue-type plasminogen activator; UK = urokinase; % NBP = percent of maximum inducible activity in normal baboon plasma; % UK plasma = percent of maximum inducible activity by UK in normal baboon plasma. 

* Nadir values.
change or rise in WBC count of these two nonsurviving baboons parallels observations made in nonsurviving humans. Finally, the mortality rates in the animal and human diseases are similar.

**Response of Nonsurvivors to Intramuscular Injection of GAS**

The two nonsurvivor baboons, which survived for only 36 and 63 hours, respectively, had positive blood culture results at 1 day and decreases in blood pressure (not shown) and neutrophil count (table 2). Figure 6 (right) shows increases in IL-6, tissue plasminogen activator, plasminogen activator-inhibitor, and CPK that were 150-, 7-, 6-, and 10-fold over baseline, respectively. In contrast, the IL-6, tissue plasminogen activator, plasminogen activator-inhibitor, and CPK concentrations of the survivor group (figure 6, left) rose only 5-, 2.5-, 2.5-, and 7-fold, respectively, over baseline during the same 2-day interval. The histopathology at the site of injection of these two nonsurvivors also differed from that observed in the survivor group.

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**Figure 2.** Histopathologic response at the site of injection of group A streptococci after 24 hours in the survivor group. *Left,* Accumulation of WBCs in the subcutaneous tissue (SC) between the dermis and underlying the muscle (M) (original magnification, ×10). Note that the fascial plane separating subcutaneous tissue and muscle was still discernable (*arrow*). *Right,* Neutrophils were the dominant cell type in this response, and they accumulated along the endomyial surface of the myofibrillar bundles (*arrows*) (original magnification, ×20). Similar cellular infiltrates were observed in the underlying muscle. Only limited necrosis and no bacterial colonies were observed in either the subcuticular or muscle tissue.

**Figure 3.** Response of markers of hemostatic system activation after injection with group A streptococci in the survivor group: plasminogen activator (*○*), fibrinogen (*□*), and fibrin degradation products (*×*). Note that fibrinogen responded as an acute-phase protein, reaching a peak concentration during stage II, whereas tissue plasminogen activator and fibrin degradation products did not reach their peak concentrations until stage III. This indicates that fibrinolytic activity occurred late and may be a part of the resolution process at the site of inoculation. Fold increase refers to the increase over baseline values.

**Figure 4.** Histopathologic responses at the site of injection of group A streptococci after 7 days in the survivor group. *Top,* Accumulation of WBC in the subcutis (SC) overlying the muscle (M) (original magnification, ×20). Gross examination of the tissue recovered from the site of injection showed complete obliteration of the plane that normally separates the subcuticular from the muscular tissue. Both tissues were involved in this response and were separated by a 3.175-mm-thick band of tissue (*bar*) in which the inflammatory response was most intense. *Bottom,* higher-power magnification (original magnification, ×40), showing that both the mononuclear cells and neutrophils had moved into the site of inflammation.

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group at 24 hours after inoculation. Figure 7 (top) shows the
junction between relatively normal and necrotic skeletal mus-

cle. No blood vessels can be seen in the area of necrosis,

whereas they can be seen in the junctional and relatively

normal areas. These vessels are packed with dark-staining

material. This material consisted of aggregated neutrophils

(figure 8). Note that the endothelium and vessel wall have a

“moth-eaten” appearance. Note also that either the tissue sur-

rounding the vessel is edematous or the cell architecture has

been lost, in contrast to adjacent myofibrillar tissues, which

appear to be intact. Finally, figure 7 (bottom) shows extensive

myofibrillar necrosis in association with the presence of nu-

merous bacterial organisms in the absence of any neutrophils.

The distribution of these organisms again follows the endomy-

sial surfaces of the myofibrillar bundle in a thin sharp line. This

distribution is similar to that of the neutrophils seen in tissue

recovered from the survivor group (figure 2). IL-6 and CPK but

not elastase/α1-antitrypsin concentrations were much higher

than those of the survivor group (figure 6). Table 2 provides

data presented in figures 1, 3, 5, and 6 as assayed rather than as

fold increases. This table summarizes the sequence of events

involving markers of phagocyte function, acute inflammatory

mediators, hemostatic mediators, chronic inflammatory medi-

ators, and markers of organ function as they appear over a

period of 19 days.

Discussion

The first stage of the response of the survivor group of

animals to im injection of GAS is dominated by the rapid
development of a flare response at the site of injection. This is

accompanied by edema, a rise in temperature, and markers of

an acute inflammatory response, including the appearance of

and peaking of IL-6, as well as elastase/α1-antitrypsin com-

plexes, together with a doubling of the neutrophil count (stages

I and II). Necrotizing fasciitis and myonecrosis occur early in

both survivors and nonsurvivors, as evidenced by a sharp rise

in CPK and loss of muscle architecture as seen by histological

evaluation. In survivors, there is histopathologic evidence of a

massive influx of neutrophils into the site of injection, involv-

ing both the subcutaneous tissue and muscle. This is followed

by an influx of lymphocytes, fibrosis, and rise in the peripheral

blood lymphocyte count and levels of C-reactive protein and

complement complexes (C4b/c) (stage III).

We concluded that the acute toxic events driven by the

β-hemolytic streptococcal organisms of stage I appear to have

been followed in stages II and III by events driven by activated

host inflammatory and hemostatic components. We believe that

this systemic inflammatory response emanating from the tissue

site of infection was the consequence of host-microbe interac-
tions that were reflected systemically by changes in the number

of lymphocytes and levels of acute-phase proteins, complement

activation products (C3b/c, C4b/c), and fibrin degradation

products. Light microscopic examination of lungs and liver

also confirms that even at 11 days after inoculation, there is a

very intense influx of inflammatory cells, characterized by

intravascular margination and aggregation. Although the cre-

tatinine and blood urea nitrogen concentrations did not rise and

the kidneys appeared normal by light microscopy, the appear-

ance of proteinaceous material in the tubules and the 50%
decline in albumin level suggests that the kidneys might also

have been involved (nephrotic syndrome).
Comparison of the above survivor group with the two non-survivor animals is instructive. First, in contrast to the survivor group, cultures of blood from nonsurvivors were positive for β-hemolytic streptococci, and their total WBC counts fell during the first 24–48 hours. This was accompanied by rises in CPK and IL-6 levels far in excess of that seen in the survivor group during this period. It should be noted that the TNF-α concentration does not rise along with IL-6 following im inoculation as it does following iv infusion of GAS. The tissue response to im injection of the streptococcal organisms also differed at 24 h. The survivors experienced a massive influx of neutrophils into the site of injection, involving both the subcutaneous tissue and muscle, whereas there was almost no neutrophil influx in the nonsurvivors. There also was little evidence of bacterial proliferation and less myofibrillar necrosis, even though the neutrophilic influx was intense in the survivors. In contrast, the lesions of the nonsurvivors showed a clear line demarcating necrotic from relatively normal skeletal muscle, and the blood vessels at this line of demarcation were choked with neutrophils.

The reasons for failure of the neutrophils to gain early, immediate access to the inoculation site range from aggregation and/or destruction of incoming neutrophils locally by toxic products of the GAS, such as seen following injection of theta toxin [48], to suppression of the production and release of neutrophils from the marrow by cytokines produced by the host in response to the infection. Other factors that impede the ability of the neutrophil to clear the infection and therefore contribute to the pathology may come into play, for example, hyperexpression of the phagocyte adherence molecule CD11b/CD18 occurring concomitantly with toxin- and cytokine-induced priming of neutrophil respiratory burst activity [49–51]. Such a mechanism could explain the massing of neutrophils within the adjacent vasculature in the nonsurvivor animals. It is also possible that local conditions created by the GAS organisms inhibited diapedesis and chemotaxis to the site of inoculation, thereby reducing the clearance of organisms from the injection site.

That the neutrophil contributes to tissue necrosis is supported by the observation that circulating elastase concentrations in serum increased during the first 48 hours of infection. However, elastase levels were not proportional to either CPK levels in serum or the degree of tissue necrosis, suggesting that tissue injury is not entirely related to the neutrophil. Direct toxicity of streptococcal factors, such as streptolysin O [52] and the cysteine protease [53], could also contribute to tissue destruction. Finally, attenuation of blood flow in these areas by intraluminal vasculitis could contribute to the rapid development of tissue destruction observed both in human cases of streptococcal TSS and in the nonsurvivor baboons in this study. Characteristics of the two nonsurvivor animals who received im injections of GAS are similar to those of animals given GAS by iv infusion as previously described by us [54]. In both cases, organisms gained access to the bloodstream, as reflected by the positive blood culture results at 1 day, and animals of both groups died. This suggests that the bacteremia is associated with lethality and that the initial local host response is important in determining survival.

### Table 3. Streptococcal toxic shock syndrome in humans: comparison with clinical and laboratory features of experimental necrotizing fasciitis in baboons.

<table>
<thead>
<tr>
<th>Clinical, laboratory, or histopathologic feature</th>
<th>Streptococcal toxic shock syndrome in humans</th>
<th>Experimental necrotizing fasciitis in baboons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance of cutaneous lesion</td>
<td>Erythema with purple discoloration, bullae</td>
<td>Erythema with purple discoloration, bullae</td>
</tr>
<tr>
<td>Fever</td>
<td>+</td>
<td>+ (nonsurvivors)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>+</td>
<td>+ (nonsurvivors)</td>
</tr>
<tr>
<td>Acute respiratory distress syndrome</td>
<td>+</td>
<td>+ (nonsurvivors)</td>
</tr>
<tr>
<td>WBC count</td>
<td>Normal or increased</td>
<td>Normal or increased</td>
</tr>
<tr>
<td>Survivors</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Nonsurvivors</td>
<td>Marked appearance of younger cells</td>
<td>Marked appearance of younger cells</td>
</tr>
<tr>
<td>Proinflammatory cytokines</td>
<td>TNF and IL-6</td>
<td>IL-6, TNF</td>
</tr>
<tr>
<td>C-reactive protein concentration</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>+</td>
<td>+ (nonsurvivors)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Fibrinogen concentration</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Serum albumin concentration</td>
<td>Decreased</td>
<td>Decreased</td>
</tr>
<tr>
<td>Creatinine concentration</td>
<td>Increased</td>
<td>Increased (nonsurvivors)</td>
</tr>
<tr>
<td>Creatine phosphokinase concentration</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Serum glutamic pyruvic transaminase concentration</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Myonecrosis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Necrotizing fasciitis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Large concentrations of streptococci at site of infection</td>
<td>+ (nonsurvivors)</td>
<td></td>
</tr>
<tr>
<td>Mortality</td>
<td>30%–70%</td>
<td>20%</td>
</tr>
</tbody>
</table>

**NOTE.** + = feature present.
Table 3 lists characteristics that are shared by this baboon model of streptococcal TSS and the human disease. Hypotension is evident early only in the nonsurvivors. Coagulopathy likewise is evident only in the nonsurvivors, and occlusive microvascular thrombi were not observed at the sites of injection and hence do not appear to play a role in the degenerative changes in the soft tissues in this model. This conclusion is supported in part by the relatively modest generation of fibrin degradation products, especially in association with the extensive inflammation and tissue damage at the site of injection. This is in contrast to the hemostatic response to iv infusion of GAS, which is associated with intense consumptive coagulopathy and generation of fibrin degradation products and plasmin/antiplasmin complexes (unpublished data). In neither baboon model, however, is this associated with an occlusive thrombotic coagulopathy. We conclude, therefore, that the coagulopathic responses of the hemostatic system in the baboon model and in humans vary and that in the baboon model, the coagulopathy is not a link in the lethal chain of events.

This primate model of myofascitis due to β-hemolytic GAS, while approximating what may occur in human disease, raises several important questions. First, by what mechanism is the influx of neutrophils inhibited in the nonsurvivors? Are neutrophil adherence and aggregation involved? To what extent are bacterial toxins and expression or activation of host neu-
trophil, platelet, and endothelial cell receptors involved? By what mechanism is the inflammatory response at the site of injection sustained over such a long period? To what extent are host mononuclear cells, complement, and acute-phase proteins, such as C-reactive protein, involved in sustaining this inflammatory response, not only at the wound site but also in lungs for as long as 11 days after im injection of streptococcal organisms? We conclude that this primate model of streptococcal myositis permits a sequential study of the various stages (natural history) of this disorder. Observation of events in this model suggests that appropriate in vitro and in vivo studies targeted specifically at factors that control influx of neutrophils into the wound site and factors (bacterial and/or host) that support a sustained myofascitis be done. These studies should include the investigation of neutrophil counter-receptor expression by the endothelium in response to fractionated streptococcal components, using cultures of mouse and primate endothelial cells and mouse models of streptococcal infection, followed by studies in selected instances making use of this primate model.

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References


