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# Systemic Injection of Group A Streptococcal Peptidoglycan-Polysaccharide Complexes Elicits Persistent Neutrophilia and Monocytosis Associated with Polyarthritis in Rats

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**The perpetuation of inflammatory changes within joints elicited by persisting, poorly biodegradable group A streptococcal cell walls (peptidoglycan-polysaccharide complexes [PG-PS]) is well documented. Chronic changes in the bloodstream induced by PG-PS have not been described previously. We demonstrated that leukocytosis occurs within 3 days after intraperitoneal injection of PG-PS and remains elevated 20 weeks later. Chronic neutrophilia, monocytosis, and lymphocytosis were observed in all experiments. Chronic changes in platelet, erythrocyte, and reticulocyte counts were not seen. The newly documented leukocytosis, lasting for months after PG-PS administration, provided a circulating pool of leukocytes that may participate in chronic inflammatory events in the joint. Although the central role of the macrophage in PG-PS-mediated inflammation has been emphasized (F. G. Dalldorf, W. J. Cromartie, S. K. Anderle, R. L. Clark, and J. H. Schwab, Am. J. Pathol. 100:383–402, 1980), the polymorphonuclear cell may be involved in periods of exacerbation of streptococcal cell wall-mediated polyarthritis. This was supported by our observations that neutrophilia and monocytosis correlate well with the degree of chronic joint inflammation.**

Bacterial cell wall fragments from group A streptococci are inflammatory and poorly biodegradable. After systemic administration, cell walls localize in tissues and persist, causing, among other things, chronic polyarthritis (5, 10, 13–15).

Substantial information exists on the ability of bacterial cell wall debris to selectively and nonselectively activate the immune system. Streptococcal cell walls are antigenic and thus elicit humoral and cellular immune responses (17, 21). These cell walls activate both the classical and alternate complement pathways (11, 26). Peptidoglycan also activates macrophages (29), which may result in the production of interleukins (9).

The clinical pattern of polyarthritis elicited by systemic administration of streptococcal cell wall fragments is related to particle size. Intermediate-sized particles of peptidoglycan-polysaccharide complexes (PG-PS) (molecular weight, $50 \times 10^6$) elicit a biphasic disease in which arthritis develops within several days, decreases in severity within 1 to 2 weeks, and subsequently develops into the chronic erosive granulomatous phase during the next several months. Thus, streptococcal cell wall-mediated polyarthritis (SCWP) has an acute and a chronic phase (5, 12). Decomplementation with cobra venom factor appreciably reduces the severity of the acute phase but not the chronic phase of SCWP. This suggests an important role for complement activation in the acute phase of SCWP (26).

A role for T lymphocytes in the chronic stages of SCWP has not been totally delineated. Schwab and co-workers (21) have shown that cell walls induce chronic arthritis in neonatally thymectomized rats, which suggests that cell-mediated immunity is not critical. Work of other investigators (24) suggests that such surgical procedures do not totally remove thymus-derived cells and notes that cell walls do not induce chronic arthritis in nude rats. Furthermore, cyclosporin (which is cytotoxic for T cells) is able to significantly inhibit chronic arthritis induced by cell walls (33).

Streptococcal cell walls activate macrophages to become cytotoxic and cytostatic for fibroblasts in tissue culture; this might also occur in vivo (29). These and other data have suggested a central role of the macrophage in mediating the chronicity of SCWP. However, since episodes of reactivation occur in arthritic tissues in which neutrophils collect even months into the disease process, polymorphonuclear cells may also be important (6).

When enzymatic digests of group A streptococcal cell walls are injected intravenously into rabbits, they induce fever and a leukocytosis which subsides within 24 h (18). Muramyl dipeptide, the smallest subunit of bacterial peptidoglycan with biological activity, also induces a transient leukopenia with a subsequent leukocytosis that lasts for several hours (30). Gram-negative bacterial lipopolysaccharides, which share many biological and chemical properties with PG, also elicit a leukopenia followed by an acute neutrophilic leukocytosis which subsides within 48 h (2). The observations presented above typify the inflammatory response seen in bacteremia in which an increase in circulating polymorphonuclear neutrophils is seen in the acute phase. There have been no previous reports of chronic neutrophilia elicited by any bacterial cell wall component.

Dalldorf et al. (6) have suggested that, although the macrophage plays the major role in PG-PS-mediated inflammation, the polymorphonuclear cell may be involved in periods of exacerbation of SCWP. Here we provide evidence to support the suggestion of Dalldorf et al. (6) by documenting that chronic neutrophilia and monocytosis last for many weeks after PG-PS administration and by showing that there is a high correlation between neutrophilia and chronic polyarthritis.

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## MATERIALS AND METHODS

**Bacterial cell wall preparation.** Streptococcal cell wall fragments were prepared as described previously (16). Briefly, *Streptococcus pyogenes* ATCC 10389 was grown in 20-liter batches of Todd-Hewitt broth (BBL Microbiology Systems, Cockeysville, Md.) and harvested with a cassette system (Pellicon; Millipore Corp., Bedford, Mass.). Cells (60 g [wet weight]) were sonicated in 45-ml batches for 90 min (Heat Systems Ultrasonics, Inc., Long Island, N.Y.) and then centrifuged for 30 min at 10,000 × *g*. The supernatants were pooled and treated sequentially with hyaluronidase (Sigma Chemical Co., St. Louis, Mo.) in 0.1 M phosphate–0.15 M NaCl (pH 5.3); DNase and RNase (Calbiochem-Behring, La Jolla, Calif.) in 0.1 M phosphate buffer (pH 7.2); papain in 0.1 M phosphate buffer (pH 7.2) containing 0.001 M cysteine, 0.001 M EDTA, and 0.05% sodium azide; and pepsin (Sigma) in 0.012 N HCl (pH 2.0). The cell walls were generally collected and washed by repeated centrifugation at 111,000 × *g* and then extracted 3 times with chloroform-methanol-water, dialyzed against water, and lyophilized. Cell wall fractions were analyzed for their carbohydrate and amino acid compositions as alditol acetates and butyl heptafluorobutyl derivatives, respectively, by gas chromatography-mass spectrometry (16). A typical PG-PS preparation consisted of rhamnose (27.2%), glucosamine (22.3%), muramic acid (6.5%), alanine (13.7%), glutamic acid (4.5%), and lysine (7.4%). Other amino acids derived from protein generally made up less than 1% of the dry weight of the fractions, with the exception of aspartic acid (1.3%).

The lyophilized cell wall preparation was sonicated at a concentration of 20 to 30 mg/ml in sterile phosphate-buffered saline (pH 7.4) for 70 min and then centrifuged at 10,000 × *g*. The amount to be injected was based on the rhamnose content, as determined by gas chromatography-mass spectrometry.

**Induction and assessment of arthritis.** Female Lewis rats from Harlan Sprague-Dawley (Madison, Wis.) (age, approximately 5 to 7 weeks; weight, approximately 100 g) were used. The animals were injected intraperitoneally (i.p.) with a sterile solution of PG-PS (3,000 μg of rhamnose, approximately 15 mg of cell wall) suspended in sterile phosphate-buffered saline. Arthritis was assessed as described by Wood et al. (32). A scale from 1+ to 4+ was used based on the degree of erythema, edema, and joint distortion of each of the four joints, to give a maximum score of 16.

**Hematology.** Modified complete blood counts, which included hematocrits and erythrocyte, leukocyte, reticulocyte, platelet, and differential counts, were performed. At various time intervals (30 min, 24 h, 72 h, 7 days, and biweekly for up to 20 weeks) after i.p. injection, the animals were anesthetized by subcutaneous injection of ketamine-xylazine and then bled via the tail vein. Blood smears were made and stained with Jenner-Giemsa (8) or Sudan black (22) for differential counts. Platelet counts were performed on a fraction of blood that was diluted in an aqueous solution of 1% ammonium oxalate. Turk solution was used to determine total leukocyte counts (8). Heparinized blood was diluted in Hematall (Fischer Scientific Co., Lacross, Ga.) to determine the erythrocyte count. The percentage of reticulocytes was determined by smears that were made from a sample of heparinized blood stained with an equal volume of new methylene blue (1). Hematocrits were measured on a microcapillary reader with a sample of heparinized blood that was spun for 2 min in a hematocrit centrifuge.

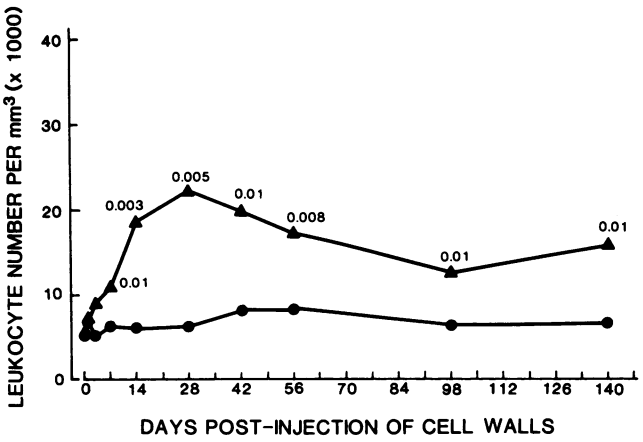


FIG. 1. Total leukocyte counts from rats injected with PG-PS (▲) and controls (●) over a 20-week period. Leukocyte counts were raised significantly in experimental animals at all time periods after 3 days. The numbers on the plot are *P* values indicating the significant difference observed between leukocyte counts in experimental and control animals at various times.

## RESULTS

**Effect of PG-PS on cellular elements of the blood.** Leukocytosis was evident within 3 days after i.p. injection of PG-PS and persisted for at least 20 weeks. Three days after injection of cell walls, the mean leukocyte count was 9,142 cells per $mm^3$ compared with a mean of 5,266 cells per $mm^3$ in control animals ($P < 0.01$). From 1 to 4 weeks the leukocyte counts in the treated animals increased dramatically, to between 11,000 and 22,000 cells per $mm^3$. These counts represented two- to threefold increases over the mean counts in the control group. At 20 weeks a mean of 16,000 cells per $mm^3$ was seen in the experimental group compared with 6,733 cells per $mm^3$ in the control group. Figure 1 shows a representative plot of the total leukocyte counts per cubic millimeter in the animals injected with PG-PS and control animals over a 20-week period.

Analysis of leukocyte differential counts revealed that the leukocytosis did not result from an equal effect on all three leukocyte elements (lymphocytes, monocytes, and neutrophils) but resulted primarily from neutrophilia. This is most readily appreciated by comparing Fig. 1 and 2 (which show absolute and differential leukocyte counts, respectively). In normal female Lewis rats, mean differential leukocyte counts were 89.8 ± 4.5% (standard deviation) for lymphocytes, 2.0 ± 1.0% for monocytes, 2.0 ± 1.0% for eosinophils, and 8.2 ± 4.1% for mature neutrophils. These cellular populations were altered in response to an i.p. injection of an arthritogenic dose of group A streptococcal cell walls. Neutrophils were elevated at all time periods from 3 days up to the longest period examined, which was 140 days after i.p. administration of streptococcal cell wall fragments. For example, at day 7, 29% of the total leukocytes were neutrophils in the PG-PS-injected animals, but 6.5% were neutrophils in control animals ($P < 0.01$). At some time periods (4 to 6 weeks) the mean neutrophil differential counts were as high as 38% of the total leukocyte counts. Even at 20 weeks, neutrophilia persisted, with 24% of the leukocytes being neutrophils in experimental animals compared with 9% in control animals. In addition to neutrophilia, an increase in the total number of large mononuclear cells (monocytes) was also noted. Monocytosis was most obvious at days 14 to 42. Leukocytes consisted of 4.5% monocytes at 14 days, 4.5% at

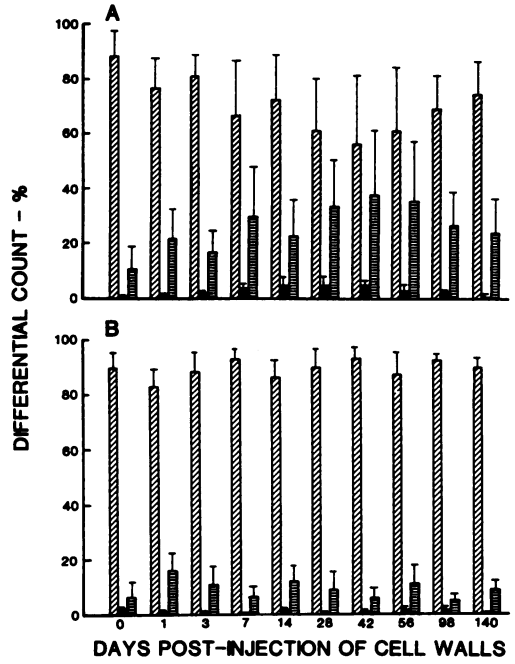


FIG. 2. The differential leukocyte counts showing percentages of monocytes (■), lymphocytes (▨), and neutrophils (▤) over a 20-week period in animals injected with PG-PS (A) and control animals (B).

28 days, and 4.8% at 42 days for experimental animals but were 1.0% or less in control animals during these same time periods. A representative plot of the differential counts for neutrophils, monocytes, and small mononuclear cells (lymphocytes) from the PG-PS-injected animals is given in Fig. 2A, and the differential counts from the control animals are given in Fig. 2B.

The magnitude of the increase in total leukocyte counts is readily appreciated when representative cell counts per cubic millimeter for the various leukocyte populations are examined. Three days after injection of PG-PS the mean neutrophil count was 1,590/mm$^3$; in controls the mean neutrophil count was 539/mm$^3$. At day 7 total neutrophil counts in experimental animals increased to 3,982/mm$^3$, but they were only 399/mm$^3$ in control animals; this was about 10-fold higher. The greatest increase in neutrophils was seen at 6 weeks after administration of cell walls, when the neutrophil count was 9,236/mm$^3$ in PG-PS-injected animals compared with 464/mm$^3$ in controls; this was about 20-fold higher. Even at 20 weeks after injection of PG-PS there were still sevenfold higher levels of neutrophils in the blood of animals injected with PG-PS (4,377/mm$^3$ in experimental animals and 608/mm$^3$ in controls). Analysis of the absolute monocyte numbers showed similar increases. Seven days after PG-PS administration, when the number of monocytes began to increase, there was a mean of 436 monocytes per mm$^3$ in experimental animals compared with only 25/mm$^3$ in controls. The greatest increase in monocyte number was at 28 days, when there was a mean of 1,266/mm$^3$ in PG-PS-treated animals compared with a mean of 35/mm$^3$ in control animals, or a 36-fold higher level in the experimental animals. Only mild lymphocytosis was observed in experimental animals, but it was observed in both the acute and chronic phases; for example, total counts at day 14 in experimental and control animals were 12,807/mm$^3$ and 5,401/mm$^3$, respectively. Representative plots of the cell counts per cubic millimeter for neutrophils (Fig. 3A), monocytes (Fig. 3B), and lymphocytes (Fig. 3C) from PG-PS injected rats compared with those from controls over a 20-week period are shown.

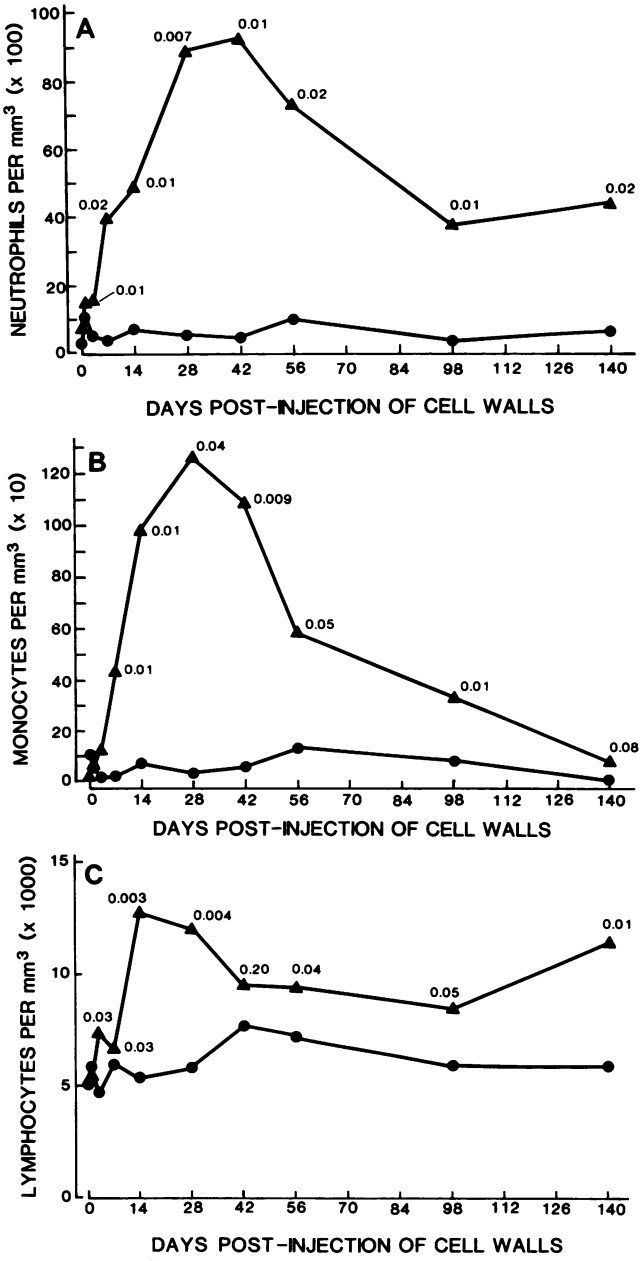


FIG. 3. A representative plot of the absolute numbers of neutrophils (A), monocytes (B), and lymphocytes (C) in experimental (PG-PS-injected) (▲) and control (●) animals. See Fig. 1 legend for an explanation of the numbers on the plot.

A typical picture of neutrophilia in the chronic phase is seen in Fig. 4A, which shows a Jenner-Giemsa-stained blood smear obtained from an animal 20 weeks after injection of PG-PS; a blood smear from a control animal is shown in Fig. 4B. During the acute stages blood from experimental animals contained many large, immature neutrophils (metamyelocytes). These cells had a kidney-shaped, heterochromatic nucleus with neutrophilic cytoplasmic granules. Metamyelocytes can be confused with mononuclear phagocytes, which

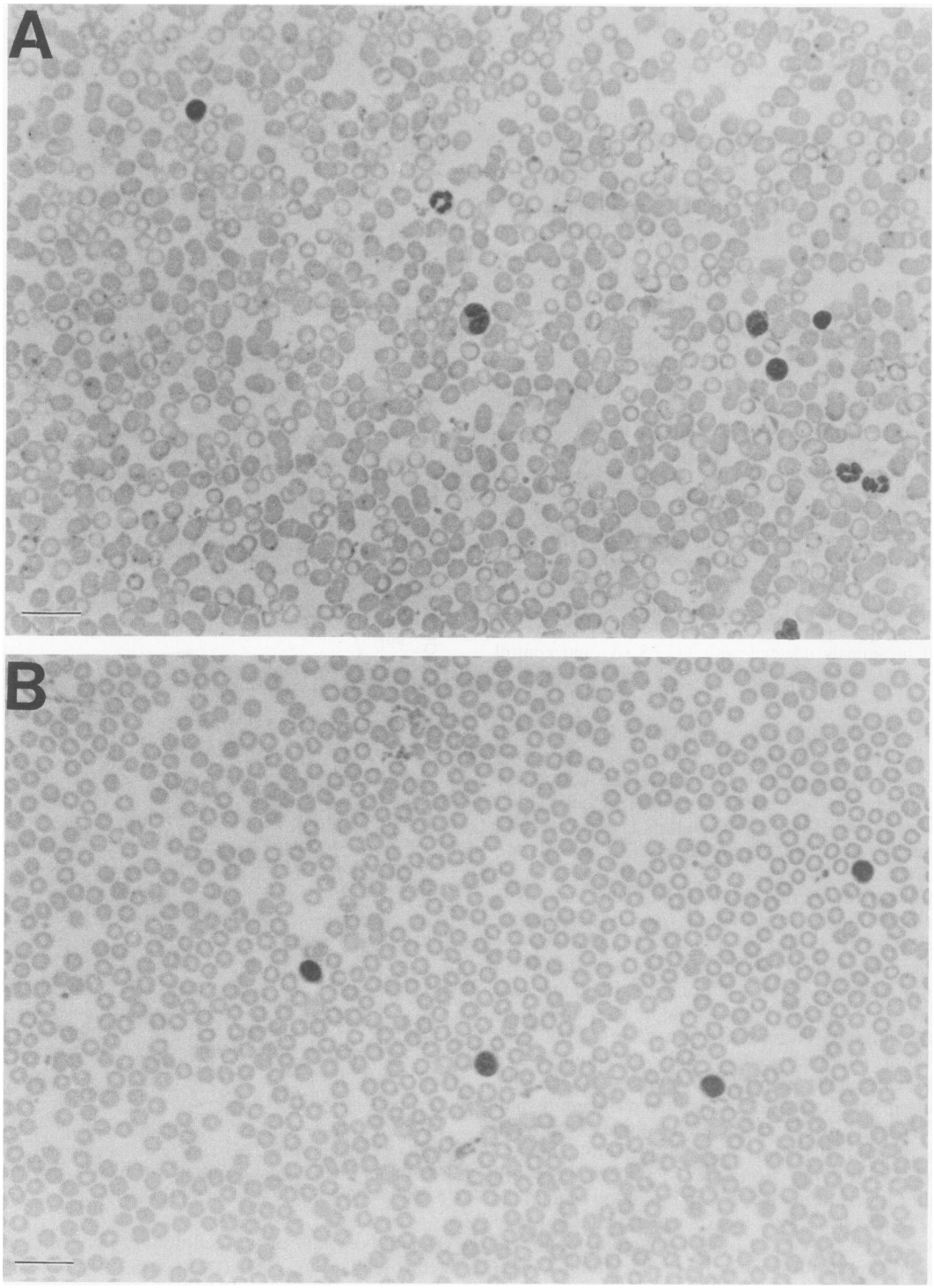


FIG. 4. Photomicrographs of a Jenner-Giemsa-stained blood smear from an experimental animal 20 weeks after injection of PG-PS (A) and from a control animal (B). Note the significantly greater number of neutrophils in panel A versus those in panel B. Bars, 20 μm.

also have an indented nucleus. A Sudan black stain was used to differentiate metamyelocytes and other immature neutrophils from monocytes; the lipid-containing granules of polymorphonuclear cells stain intensely black. A comparison of differential counts of blood smears, stained with Jenner-Giemsa or Sudan black, from experimental and control animals 7 days after administration of streptococcal cell walls is shown in Table 1. The results obtained with both stains were similar, confirming that the leukocytosis induced by PG-PS is primarily caused by neutrophilia.

In all experiments, platelet counts fell below control levels within 24 h after PG-PS injection but returned to control

TABLE 1. Differential leukocyte counts of blood smears from animals 7 days after injection of PG-PS and blood smears from controls

| Animals and animal no. | Counts of the following as a percentage of total leukocytes[a]: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lymphocyte | | Monocyte | | Neutrophil | | Eosinophil | |
| | J-G | SB | J-G | SB | J-G | SB | J-G | SB |
| Injected with PG-PS | | | | | | | | |
| 1 | 59 | 47 | 6 | 5 | 32 | 48 | 1 | 0 |
| 2 | 64 | 55 | 0 | 2 | 35 | 43 | 1 | 0 |
| 3 | 62 | 65 | 7 | 3 | 30 | 32 | 1 | 0 |
| 4 | 69 | 62 | 3 | 2 | 28 | 36 | 0 | 0 |
| 5 | 83 | 86 | 1 | 1 | 16 | 13 | 0 | 0 |
| 6 | 61 | 68 | 2 | 4 | 37 | 28 | 0 | 0 |
| Mean | 66 | 64 | 3 | 3 | 30 | 33 | 1 | 0 |
| Control | | | | | | | | |
| 1 | 85 | 90 | 2 | 2 | 12 | 8 | 1 | 0 |
| 2 | 88 | 80 | 1 | 1 | 10 | 19 | 1 | 0 |
| 3 | 79 | 85 | 4 | 1 | 16 | 14 | 1 | 0 |
| 4 | 86 | 90 | 2 | 5 | 11 | 5 | 1 | 0 |
| 5 | 83 | 77 | 1 | 2 | 16 | 21 | 0 | 0 |
| 6 | 77 | 83 | 1 | 2 | 20 | 15 | 2 | 0 |
| Mean | 83 | 84 | 2 | 2 | 14 | 13 | 1 | 0 |

[a] J-G, Jenner-Giemsa; SB, Sudan black.

values within 7 days and remained there throughout the duration of the experiments (up to 98 days, which was the longest time period examined). Table 2 shows the ratios of the total platelet counts of experimental animals to those of control animals at various time periods in a representative experiment. Note that at only 24 h was there a significant difference ($P < 0.0002$) in the experimental versus the control animals. PG-PS had no effect on the erythrocyte counts or on the hematocrits, nor did we note any effect on reticulocyte counts.

**Effect of particle size on cellular elements of the blood.** In order to determine whether the size of cell wall particles had an effect on the various systemic changes noted above, two groups of rats were treated; one was injected with a suspension of small PG-PS particles in PBS (similar to 100S), and another was injected with a suspension of intermediate-sized PG-PS particles (similar to 100P), which were prepared as described previously (12). Some particles remain in the supernatant upon centrifugation at 100,000 × *g* (100S), while other particles form a pellet (100P). In agreement with our earlier work, the smaller particles induced more severe acute

TABLE 2. Ratios of the total platelet count of animals injected with PG-PS to those of control animals over a 98-day period

| Time | Experimental/control ratio |
|---|---|
| 30 min | 0.857 |
| 1 day | 0.522[a] |
| 3 days | 1.000 |
| 7 days | 1.200 |
| 14 days | 1.092 |
| 28 days | 1.066 |
| 42 days | 1.033 |
| 56 days | 1.089 |
| 98 days | 1.109 |

[a] One day after PG-PS administration the *P* value was <0.0002. This was found in each of three experiments. At all other times there was no significant difference between control and experimental groups.

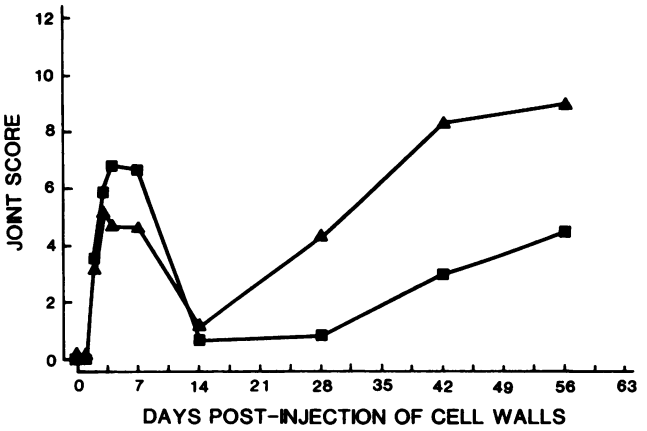


FIG. 5. Comparison of arthritis induced by small (■) and intermediate-sized (▲) PG-PS particles over an 8-week period. No arthritis was seen in control animals injected with phosphate-buffered saline alone.

arthritis, while the intermediate-sized particles induced more severe chronic arthritis (Fig. 5). Both small and intermediate-sized cell wall particles induced a chronic neutrophilia (Fig. 6A) and monocytosis (Fig. 6B). There was, however, no significant difference at any time period between the degree of neutrophilia or monocytosis induced by small and

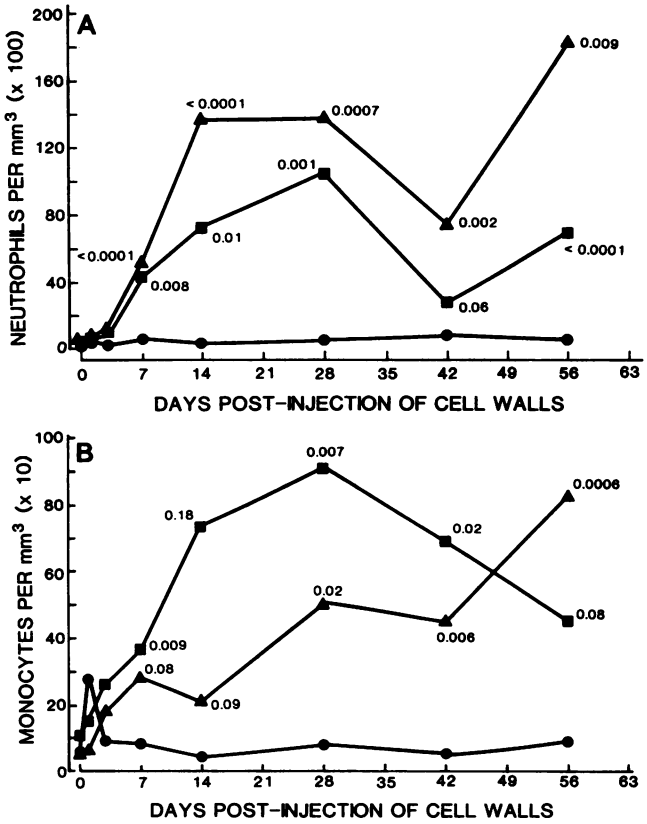


FIG. 6. Comparison of the effect of small (■) versus intermediate-sized (▲) PG-PS particles over an 8-week period on neutrophils (A) and monocytes (B). Control animals were injected with PBS alone (●). See Fig. 1 legend for an explanation of the numbers on the plot.

TABLE 3. Effect of size of cell wall fragments on total leukocyte counts over an 8-week period

| Time | Experimental/control ratio after injection of[a]: | |
|---|---|---|
| | Small particles | Intermediate-sized particles |
| 30 min | 1.060 | 1.302 |
| 1 day | 0.732 | 0.736 |
| 3 days | 0.814 | 0.871 |
| 7 days | 1.377 | 1.656 |
| 14 days | 2.933 | 3.704 |
| 28 days | 2.597 | 2.933 |
| 42 days | 1.916 | 2.075 |
| 56 days | 3.425 | 3.774 |

[a] The experimental leukocyte counts were significantly raised at all time periods later than 3 days postinjection of PG-PS; $P$ values were always <0.01.

intermediate-sized particles. Table 3 shows the total leukocyte counts in control and experimental animals. Both small and intermediate-sized particles also induced an acute thrombocytopenia. The ratio of platelet counts in PG-PS-treated animals to those in control animals over a 98-day period for intermediate-sized PG-PS particles is shown in Table 2.

**Correlation of leukocyte counts with joint scores.** On days 3, 7, and 42 after PG-PS injection, linear regression analyses of the joint scores versus lymphocyte, monocyte, and neutrophil counts were performed. The regression coefficients and $P$ values are presented in Table 4. Neutrophil and monocyte counts showed a highly significant correlation with joint score on days 7 and 42. In contrast, there was no correlation of the lymphocyte counts with the joint scores at any time period. The strongest correlation of joint score was with neutrophil counts on day 42, with an $r$ value of 0.88 and a P value of less than 0.0001. Figure 7 shows the relationship of the arthritic index to the neutrophil counts at days 3 (Fig. 7A), 7 (Fig. 7B), and 42 (Fig. 7C).

## DISCUSSION

We showed that group A streptococcal cell walls induce a chronic neutrophilia and monocytosis in Lewis rats which correlates with chronic joint inflammation.

The acute effects of various bacterial cell wall components on leukocytes circulating in blood and collecting in pools at the margins of vessels are well known (2, 4, 18). For example, gram-negative endotoxin elicits a biphasic response consisting of leukocytopenia followed by leukocytosis which subsides within a few hours (4). This increase is due to the release of granulocytes from the bone marrow into the peripheral blood (4). Muramyl dipeptide, a small subunit of peptidoglycan which is readily biodegradable (20), also induces an acute lymphocytosis that lasts 48 h in mice (31) and that correlates with the proliferation of bone marrow progenitor cells. In sharp contrast, we observed a severe, chronic neutrophilic leukocytosis. As late as 140 days after PG-PS injection there was an increase in the total number of circulating neutrophils in animals injected with PG-PS. There was also a chronic monocytosis.

Following the administration of endotoxin, a series of events occurred which ultimately led to leukocytosis. One possible scenario for the neutrophilia and monocytosis induced by streptococcal cell walls may be similar to the one for the leukocytosis induced by endotoxin. Interleukin-1 may be produced by macrophages that are exposed to cell walls. Interleukin-1 may then induce fixed bone marrow

TABLE 4. Correlation between joint scores and leukocytes counts[a]

| Cell type | Correlation coefficient and probability on the following days after PG-PS injection: | | | | | |
|---|---|---|---|---|---|---|
| | 3 | | 7 | | 42 | |
| | $r$ | $P$ | $r$ | $P$ | $r$ | $P$ |
| Neutrophil | 0.40 | 0.03 | 0.62 | 0.009 | 0.88 | <0.0001 |
| Monocyte | 0.47 | 0.01 | 0.58 | 0.002 | 0.60 | 0.008 |
| Lymphocyte | 0.17 | 0.40 | 0.26 | 0.210 | 0.13 | 0.61 |

[a] Linear regression analysis was by least squares.

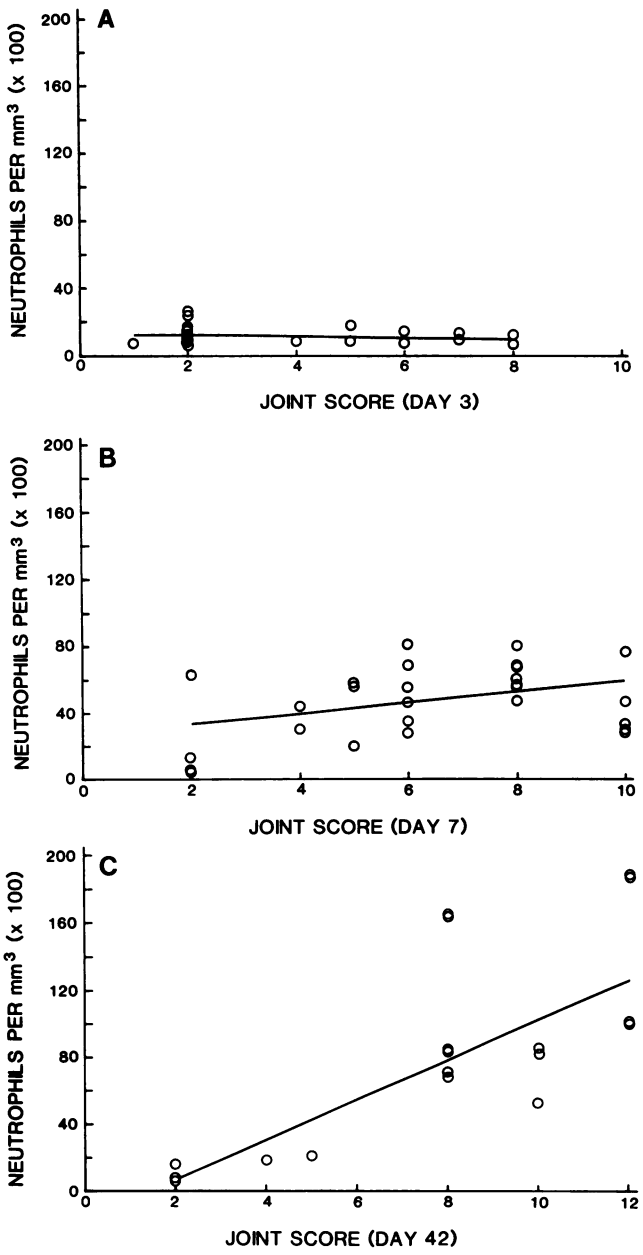


FIG. 7. Relationship of the degree of arthritis to the neutrophil count on day 3 (A), day 7 (B), and day 42 (C). Lines were fitted by least-squares analysis.

endothelial cells and fibroblasts to produce colony-stimulating factor for granulocytes and monocytes. Colony-stimulating factor for granulocytes and monocytes, in turn, may stimulate the formation of granulocyte and monocyte colonies which produce large numbers of neutrophils and monocytes that are eventually released from the bone marrow into the general circulation. These circulating cells may participate in events which occur during the chronic phase of PG-PS-mediated arthritis (7, 23, 28).

The group A streptococcal cell wall is resistant to degradation (10, 14, 27). The persistence of PG-PS in tissues might act as a sustained stimulatory source for leukocyte modulators, including interleukin-1. Certain complement by-products are chemotactic for neutrophils, and PG-PS does activate the alternate complement pathway which correlates with the acute inflammation elicited by PG-PS (26). Complement activation products could also contribute to the chronic neutrophilia seen in PG-PS-injected animals.

The size of the cell wall polymer is important in the severity and clinical course of the arthritis that is induced (3, 12). Soluble PG-PS fragments (molecular weight, $<5 \times 10^5$) elicit an acute edema in the joints of rats. Small particles (molecular weight, $5\times 10^6$; 100S) cause an acute arthritis that develops within a few days. Intermediate-sized particles (molecular weight, $50 \times 10^6$; 100P) cause a moderate early inflammation and the most severe chronic erosive joint lesions. Large particles (molecular weight, $500 \times 10^6$) induce a polyarthritis that takes several weeks to develop. We also noted that small and intermediate-sized particles induced an acute thrombocytopenia and chronic neutrophilia and monocytosis. Bacterial cell wall components mediate the lysis and clumping of platelets involving the classical complement and clotting pathways (19, 25). Chronic neutrophilia and monocytosis induced by PG-PS particles of any size have not been reported previously.

We have described here several new features of the SCWP model. First, inflammation is not limited to peripheral tissues, but rather there is a persistent increase in the levels of circulating cellular components of the blood. These chronic changes affect monocytes, lymphocytes, and neutrophils. However, neutrophilia induced by PG-PS most strongly correlates with the arthritic index at times during the chronic phase of inflammation. While this report was in preparation, J. H. Schwab also noted that PG-PS induces monocytosis (seminar presentation, 88th Annual Meeting of the American Society for Microbiology, Miami Beach, Fla., 8 to 13 May 1988).

In chronic tissue lesions, it is common to see periods of acute inflammation with neutrophil infiltration and fibrin deposition superimposed on the characteristic mononuclear cell infiltrate (6). However, it is rare to find elevated neutrophil counts in the systemic circulation at times during the chronic phase of inflammation. Circulating neutrophils are considered to be expendable or short-lived cells since their function is to be completed shortly after they enter tissues (2, 4). The PG-PS-induced neutrophilia and monocytosis may provide a large, readily accessible, and continuously available source of polymorphonuclear cells (and monocytes) that may be recruited to aid in the clearance of bacterial components and tissue debris in chronic lesions of joints. Once recruited, such cells could be activated to produce inflammatory mediators, as is known to occur in rheumatoid arthritis (7). Results of this study support a role, as suggested previously (6), for the neutrophil and monocyte in the exacerbation and persistence of the chronic lesions seen in streptococcal cell wall-mediated arthritis (6). It is tempting to speculate that the monocyte is the major cell involved in persistent tissue injury, but the neutrophil plays an important role in exacerbations of inflammation.

## ACKNOWLEDGMENTS

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