Effect of Penicillin on the Humoral and Cellular Immune Response Following Group A Streptococcal Pharyngitis

R. A. Zimmerman, P. H. Klesius, D. H. Krushak and J. H. Mathews*

ABSTRACT

The effect of oral and parenteral penicillin on the development of cellular and humoral immune responses in chimpanzees infected with group A streptococcal M-types 1, 5 and 12 was investigated. The interrelationship between type-specific antibody response and enhancement of phagocytic competence of polymorphonuclear neutrophils was documented. Penicillin depressed or suppressed type-specific antibody response depending on the mode and dose of administration, probably because of its effect on the streptococci responsible for antibody stimulation. Penicillin was not demonstrated to have a direct effect on phagocytic ability in vitro. Therefore the primary effect of antibiotic therapy is the indirect relationship to suppression or inhibition of type-specific antibody response to M-protein which results in a diminution of phagocytic competence.

RÉSUMÉ

Cette expérience visait à étudier l'effet de l'administration orale et parentérale de pénicilline sur le développement de la réponse immunologique cellulaire et humorale, chez des chimpanzés infectés avec les types M 1, 5 et 12 des streptocoques du groupe A. On démontra la relation entre la réponse immunologique propre à chacun de ces types et l'augmentation du pouvoir phagocytaire des neutrophiles. La pénicilline diminua ou supprima la réponse immunologique propre à chacun des types, selon la dose et la voie d'administration de cet antibiotique, probablement à cause de son effet sur les streptocoques responsables de la stimulation d'anticorps. On ne démontra pas que la pénicilline exerçait une action directe sur le pouvoir phagocytaire, in vitro. Par conséquent, l'effet primordial de l'antibiothérapie se traduit par une relation indirecte vis-à-vis la suppression ou l'inhibition de la réponse immunologique envers la protéine M, propre à chacun des types de streptocoques; ce phénomène entraîne une diminution du pouvoir phagocytaire.

The appearance of type-specific antibody (TSA) following group A streptococcal pharyngitis is one factor in the development of natural immunity. The opsonin action of TSA has been described (7). Therefore, a combination of humoral and cellular responses measured by the development of TSA and enhanced phagocytic activity of polymorphonuclear neutrophils (PMN) is probably of primary importance. The effect of antibiotic therapy on these immune mechanisms has been questioned (1, 4).

*Fort Collins and Phoenix Laboratories, Ecological Investigations Program. Center for Disease Control. Health Services and Mental Health Administration, Public Health Service, United States Department of Health, Education, and Welfare.

Formerly Chief, Streptococcal Disease Section, Fort Collins Laboratories, Fort Collins, Colorado (Zimmerman). Formerly Assistant Chief, Streptococcal Disease Section, Fort Collins Laboratories; presently with USDA Regional Parasite Research Laboratory, Auburn, Alabama (Klesius). Chief, Animal Experimentation Unit, Phoenix Laboratories, Phoenix, Arizona (Krushak). Chief, Immunology Unit, Streptococcal Disease Section, Fort Collins Laboratories (Mathews).

Reprint requests: J. H. Mathews, Fort Collins Laboratories, P.O. Box 2087, Fort Collins, Colorado 80521.

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Several investigators have suggested that penicillin has a suppressive or depressive effect on humoral antibody against several streptococcal antigens (6, 10). Furthermore, the dosage of penicillin given appears to relate directly to the amount of antibody production observed. In previous controlled studies of streptococcal pharyngitis in chimpanzees we have described total depression of TSA response following adequate dosages of parenteral benzathine penicillin (10). To our knowledge, similar studies on the effect of antibiotic therapy on phagocytic competence against group A streptococci have not been undertaken. This paper describes our investigation on the effects of parenteral and oral penicillin on the humoral and cellular immune responses observed in chimpanzees following induced pharyngeal infections with group A, M-types 1, 5 and 12.

Animals — Eight healthy chimpanzees (*Pan troglodytes*) weighing 30-50 kg were selected. They had been previous participants in our streptococcal research but had had no group A experience within a year or more previous to this study. The influence of prior infections on the observations made in this study is unknown.

Group A streptococci — M-types 1, 5 and 12 were isolated from children with streptococcal pharyngitis who were participants in our epidemiological studies in Colorado (12). Each serotype was a strong M-protein producer and would grow in human blood. Previous studies in our laboratories had shown that these serotypes would produce clinical pharyngitis in chimpanzees which closely resembles the disease in man (6, 10).

Inocula for the chimpanzees were prepared according to our previously tested procedures (10). To induce infection, animals were inoculated intranasally with 0.5 ml (2.7×10^7 colony forming units/ml) of the broth culture into each naris. The primates were anesthetized before each procedure with phencyclidine hydrochloride at recommended dosage.

Clinical material — Pharyngeal swabs and blood specimens were obtained weekly before and during the study period. The swabs were placed in individual tubes containing sterile silica gel, mailed to Fort Collins and the streptococci were grouped and typed as previously described (9).

Blood specimens were collected with heparin as an anticoagulant (143 USP units 17 ml) by femoral venipuncture. The bloods were shipped by air in ice cooled containers and received within 48 hours.

Clinical records were kept on all animals. Signs and symptoms of pharyngitis were noted. Rectal temperatures were taken. Total and differential leucocyte counts were obtained.

Criteria for streptococcal infection — Several criteria were used: 1) recovery from the pharynx of the same M-typeable streptococci as the inoculum, 2) levelopment of clinical signs and symptoms of pharyngitis and 3) elevation of rectal temperatures and leukocytosis.

Protocol for experiments — The experimental plan consisted of a preinfection period of at least two weeks. Colonization of the animals as described was done at day 15. Within seven days following fulfillment of criteria for infection the antibiotic regimes were begun.

Oral penicillin treatment, potassium phenoxymethyl penicillin, was given in a strawberry flavored water mixture (8 ounces of jello with water 1:5). The chimpanzees had been trained to drink the cocktail prior to the study. Each treated animal was given one million units of medication daily with the dose being divided equally every 12 hours for ten days.

A single dose of parenteral penicillin, 1.2 million units benzathine penicillin G, was given.

Both treatment regimens successfully eradicated the organism in each infected animal.

One cohort of three chimpanzees was colonized with serotype M-1, another with M-5 and a third cohort of only two chimpanzees with M-12. One chimpanzee in each cohort received oral penicillin, one parenteral penicillin and one remained untreated. An untreated control was not included in the M-12 infected cohort.

The postantibiotic period represented an interval of approximately three to four weeks following the completion of penicillin therapy.

Antibody response — TSA responses against streptococcal M-types 1, 5 and 12 were measured by indirect hemagglutination assay, previously described (11).

Phagocytosis — PMN were obtained from the heparinized blood samples according to the following method: The bloods were

centrifuged at 1000 x G for 15 minutes at 4°C. After removal of the cell free plasma the blood was gently mixed with 3% gelatin in phosphate buffered saline (PBS), pH 7.6. The mixture was incubated at 37°C until sedimentation of erythrocytes was apparent. The top layer of the mixture containing the PMN was removed. These cells were placed in a sterile silicone coated tube and held in ice. The enriched white blood cell preparation containing PMN was washed three times with 10% bovine serum albumin in PBS. The concentration of leukocytes was adjusted to approximately 14,000/ml by the use of a leukocyte counting chamber.

The phagocytic activity of PMN was determined by a modification of a procedure previously described (5). The streptococci were grown for 18 hours in Todd-Hewitt broth at 37°C and washed three times in PBS. The approximate number of colony forming units/ml in the standardized suspensions was 1.8×10^7. Standardization was accomplished by adjusting in PBS their optical density to 0.2 at 540 nm. The ratio of streptococci to PMN was about 250 to 1. The phagocytic index (PI) was calculated by counting the number of bacteria engulfed by each of 50 randomly selected PMN per smear. The percentage of PMN that were phagocytically active was calculated.

The clinical signs and symptoms of acute group A streptococcal pharyngitis were identical to those previously described (5). Infection was achieved in all cases with a single inoculating dose. During treatment with either antibiotic regime signs became less acute. Posttreatment temperatures persisted up to 2°F above normal for three or four days. Purulent tonsillitis, pharyngeal redness and swelling were noted early in the infection but presence of exudate terminated abruptly after onset of therapy. Although swelling decreased after treatment pharyngeal redness persisted. White blood cell counts increased as much as threefold after onset of infection and remained somewhat elevated during the course of treatment. Differential counts showed an increased ratio of PMN.

Examination of urine by Hemacombistix showed evidence of mild proteinuria and lower pH after infection and during treatment.

TSA responses against M-types 1, 5 and 12 were determined for each of the weekly serum samples collected during the seven weeks of the experiment. These antibody responses typically rise between 12 and 14 days after group A infection (10). Homologous TSA responses were noted against the appropriate infecting M-type in the cohort receiving oral penicillin treatment. The untreated chimpanzee in this series showed a slightly earlier response and one of significantly greater magnitude. Oral penicillin apparently has two effects: 1) a slight delay in initiation of TSA response, and 2) a depression in the magnitude of homologous TSA titer.

The effect of parenteral penicillin on development of TSA titers against M-types 1, 5 and 12 was observed. In two chimpanzees homologous TSA response was completely suppressed after infections with M-1 and M-5. The primate infected with M-1 had a significant preinfection M-1 titer. However, the expected substantial anamnestic response did not occur. The untreated control animal showed normal homologous TSA response after M-5 challenge.

Phagocytic activity against M-types 1, 5 and 12 by treated and control chimpanzees was determined. In general, the phagocytic activity of the PMN from untreated primates rises after challenge and continues to increase as long as the individual remains colonized. However, in the treated chimpanzees there was a rather abrupt decrease in phagocytic ability after initiation of either mode of therapy. We noted that this diminished activity was more marked in those animals receiving parenteral penicillin.

In order to determine whether penicillin had a direct effect on PI *in vitro* an additional experiment was performed. Phagocytic activity against M-1 cells was not altered by addition of increasing amounts of Pen-Vee-K to plasma.

The natural development of TSA in untreated chimpanzees following infections with M-types 1, 5 and 12 was observed in this study and was similar to our earlier observations (10). Detection of TSA ordinarily is possible between 12 and 14 days postcolonization. However, if there are existing high levels of TSA of a particular serotype there may be a delay or even a suppression of homologous TSA response. The phenomenon may be dependent upon the similarity of the antigenic relationship between the serotypes that caused the previous response and the type responsible

for the antigenic challenge. Several investigators have described cross-reactivity between group A serotypes (2, 3, 8). Some of these are one-way crosses. Without considering antibiotic regime, it was noted that rises in existing M-1 TSA occurred in two primates following M-5 challenge. Similar change was not observed following M-12 challenge. M-5 challenge did not alter significantly M-12 TSA titers in two animals. Similarly, M-1 challenge did not alter M-12 TSA. This suggests that without consideration of other immunological or treatment effects a challenge by a particular M serotype might be moderated if appropriate cross-reaction TSA were present in sufficient quantities.

The effect on homologous TSA response with oral and parenteral penicillin therapy but without regard to existing homologous or heterologous TSA was observed. Following oral penicillin treatment, all primates responded with appropriate homologous TSA but at levels substantially lower than control animals. Therefore, there was a depression of antibody development. Contrarily, parenteral penicillin inhibited homologous TSA response in two chimpanzees. In addition, an expected anamnestic response was not observed in one primate following homologous challenge. Therefore, we conclude that the differences in TSA response following oral or parenteral penicillin result from more prompt eradication of the organism due to the higher immediate blood levels that are obtained with the use of parenteral benzathine penicillin G.

Parenteral penicillin appeared to depress phagocytic activity more than the oral regime without consideration of TSA. Furthermore, our *in vitro* experiments demonstrated no direct effect of penicillin on phagocytic ability of PMN. Therefore, a consideration of the effect of penicillin therapy must take into account both TSA development and cellular response. Oral penicillin resulted in only a slight suppression of phagocytic activity because the chimpanzees in this group all responded concurrently with production of homologous TSA. This was especially apparent when phagocytic activity closely paralleled simultaneous TSA response. The more drastic effect of parenteral treatment on phagocytic index values is shown in primates in which no homologous TSA response occurred. In one chimpanzee with high levels of homologous TSA before infection by the same serotype (M-1) only a gradual decrease in phagocytic activity was observed.

Finally, one other observation is worth comment. When a chimpanzee was challenged by any M serotype, nonspecific increase in phagocytic competence was noted. PMN activity against all heterologous group A M-types used in the study was enhanced, regardless of the challenging serotype. Parallel experiments using bovine serum albumin instead of homologous plasma show similar increased sensitivity of PMN to all serotypes. This may represent previous sensitization of PMN to earlier challenge of these chimpanzees by group A streptococci a year or more previous to this experiment. Phagocytic activity continued to increase only when TSA response developed. It is concluded that the primary effect of pencillin therapy, possibly through its direct effect on the organism, involves the depression or suppression (depending on regime) of TSA. This, in turn, is directly correlated with enhancement of phagocytic activity of PMN.

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