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FORMATION OF ANTIBODIES TO NATIVE DNA IN RATS AFTER ADMINISTRATION OF NATIVE DNA TREATED WITH THE XANTHINE-XANTHINE OXIDASE SYSTEM

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The injection of native (double-stranded) deoxyribonucleic acid treated with the xanthine-xanthine oxidase system and emulsified with complete Freund's adjuvant into rats over a prolonged period of time induces the formation of antibodies to double-stranded DNA. The titer of antibodies was determined by an enzyme-linked immunosorbent assay (ELISA) in sera from treated animals. Control experiments using untreated native DNA or phosphate buffered saline likewise emulsified with Freund's Adjuvant showed only insignificant increases in titers of the antibody.

Key words: antibodies, deoxyribonucleic acid, xanthine, xanthine oxidase

INTRODUCTION

Human as well as murine Systemic Lupus Erythematosus (SLE) is characterized by the specific presence of antibodies to double-stranded DNA in serum[1]. The occurrence of this particular kind of antibody is routinely used as a diagnostic criterion to verify that a patient suffers from the disease[2]. During periods of remission of the illness the titer of antibody significantly declines[3].

Attempts to induce the formation of antibodies to native DNA by injection of the substance into normal experimental animals have so far proven unsuccessful; this in contrast to the ease with which antibodies to denaturated (heat- or UV-light-treated) DNA are formed in animals after the administration of such modified DNA[4,5]. It is not known why native DNA is not antigenic despite the fact that antibodies to the substance are found in high titers in individuals with SLE. A reasonable assumption may be that the DNA must be chemically modified in vivo in order to attain immunogenic properties[6].

During recent years an increasing amount of evidences has accumulated in literature that free oxygen radicals produced during cell metabolism, i.e. at phagocytosis, may play an important role in the pathogenesis of rheumatic disease including Systemic Lupus Erythematosus[7,8,9,10]. It is well established that oxygen derived free radicals such as the superoxide anion and the hydroxyl radical in vitro exert profound effects on various macro-molecules such as deoxyribonucleic acid[11]. In this communication we report that native DNA treated with a superoxide anion generating

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system (xanthine-xanthine oxidase) appears to be transformed into an immunogenic state.

EXPERIMENTAL PROCEDURES

Modification of native DNA

150 mg of calf thymus DNA were dissolved in 15 ml of phosphate buffered saline (PBS) and then extensively dialyzed against 0.03 M acetate buffer pH 4.8 at +4°C. In order to remove all single-stranded DNA the dialyzed solution was incubated with 5000 units of S1 nuclease for 4 hours at 37°C. The DNA solution was again dialyzed against PBS to remove all low molecular weight reaction products formed during the S1 nuclease incubation step. One half of the dialyzed solution free from single-stranded DNA was incubated with xanthine (5.10^{-4} M) at 5 units of xanthine oxidase enzyme for 3 hours at pH 7.4 and room temperature. The remaining portion of the DNA solution was similarly incubated with xanthine and heat-inactivated xanthine oxidase enzyme. Both solutions were then dialyzed against PBS to remove unreacted xanthine and enzymically formed hypoxanthine. Spectrophotometric analysis at 260 nm showed a DNA concentration of 1.4 mg per ml in both solutions. Determination of the T_M-value of the treated DNA solutions showed the DNA to be in double-stranded state.

Treatment of experimental animals

Female Wistar rats aged 5 months were used for intramuscular injections of the DNA solutions prepared as described above. The solutions were emulsified with an equal volume of Complete Freund's Adjuvant immediately before use. Phosphate buffered saline similarly emulsified with Freund's Adjuvant was used as a control. Seven rats were treated with each solution and were injected twice a week with 0.15 ml of the respective emulsified solution. After 4 weeks of treatment blood was collected from each animal by heart puncture using heparinized needles. Serum was prepared from each blood sample and stored at −70°C until use.

Determination of anti-DNA antibodies

Relative concentrations of antibodies to native DNA in the various sera were determined by conventional enzyme-linked immunosorbent assay (ELISA)[12].

Materials

Calf thymus deoxyribonucleic acid Type I; S1 Nuclease Type III; xanthine oxidase Grade IV; Complete Freund's Adjuvant; bovine gamma globuline Fraction II; Sigma 104[R] phosphatase substrate and sheep anti-rat IgG alkaline phosphatase conjugate were all purchased from Sigma chemical company, St. Louis, Mo., USA. Bovine serum albumine was purchased from United States Biochemical Corp., Cleveland, Ohio, USA. All other chemicals used were of ordinary analytical grade purity.

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TABLE
Relative concentrations of antibodies against native DNA in sera from rats injected with xanthine-xanthine oxidase treated DNA

Serum from rats treated with	Relative concentrations of antibodies expressed as $O.D._{405\ nm}/20$ min.[a]
1. PBS	0.327 ± 0.101 S.D.
2. Untreated DNA	0.233 ± 0.100 S.D.
3. Treated DNA	1.314 ± 0.238 S.D.

[a] Mean and standard deviation of 7 samples of serum.

RESULTS AND DISCUSSION

According to the Table above it is apparent that treatment of native, double-stranded deoxyribonucleic acid with the superoxide anion generating system xanthine-xantine oxidase enzyme transforms the DNA into an immunogenic state. The rather insignificant titers of antibodies to double-stranded DNA found in sera from animals treated with unmodified DNA or with PBS can probably be referred to as non-specific antibody interactions with the DNA-coating of the microtiter wells. The results seem to support recent ideas of the role played by free oxygen radicals in the development of Systemic Lupus Erythematosus[7]. Even if it is not unequivocally shown in this paper that the formation of antibodies is due to free oxygen radical modification of the DNA molecules, it is hard to believe that the xanthine oxidase enzyme itself would have such antibody inducing ability especially considering the very small amounts of the enzyme used. It is more conceivable that the DNA is modified by the generated superoxide anions or some species derived therefrom in such a way that it becomes immunogenic. Significant titers of anti-DNA antibodies are found in sera from humans as well as from mice suffering from this disease. Systemic Lupus Erythematosus is generally regarded as an immunologically determined chronic inflammatory disease but it might be speculated in that as a consequence of the "oxygen burst" of activated phagocytes a certain amount of formed free oxygen radicals can escape to the extracellular environment outside the inflamed site and thus interact with occurring "waste" DNA and modify this to become antigenic and give rise to antibodies to double-stranded DNA so specific for Systemic Lupus Erythematosus.

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