Inhibitory effect of syphilitic rabbit serum on DNA synthesis in rabbit cells in vitro

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SUMMARY A previously described toxic factor associated with Treponema pallidum (Nichols) and found in extracts of syphilitic rabbit testes has now also been detected in syphilitic rabbit serum. The toxic factor, which inhibits DNA synthesis in baby rabbit genital organ (BRGO) cells in vitro, is present in rabbit serum up to 30 days after infection with T pallidum.

Introduction

Treponema pallidum (Nichols strain) and extracts of syphilitic rabbit testes inhibit macromolecular (DNA, RNA, and protein) synthesis in rabbit cells in vitro. This finding was one of very few reports of direct toxicity associated with T pallidum. Fitzgerald et al and Oakes et al have also reported mammalian cell toxicity associated with T pallidum. The relevance of these reports is that tissue destruction in early syphilis may be due to direct treponemal toxicity rather than to the host's inflammatory and immune response to the treponeme as was previously assumed.

We report a toxic factor that inhibits the synthesis of DNA in rabbit cells in vitro and is detectable in serum from syphilitic rabbits up to 30 days after infection with T pallidum. Serum from rabbits immunised with heat killed T pallidum was not toxic.

Materials and methods

RABBIT SERUM SAMPLES
Three rabbits were injected with viable T pallidum organisms $5 \times 10^5$/testis. The rabbits were bled before infection to provide normal rabbit serum and periodically from five to 150 days thereafter. Control (sham infected) rabbits were injected with heat killed (at 56°C for 30 minutes) T pallidum organisms $5 \times 10^5$/testis and bled similarly. Individual samples of serum were stored separately at $-70°C$ until all were ready for experimental use. All serum samples were sterilised by membrane filtration (Millipore 0.45 μm) before coincubation with tissue culture cells.

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Preparation of T pallidum
T pallidum organisms were harvested from infected rabbit testes within two days of the development of orchitis. The testes were cut into small slices that were transferred to a 150 ml bottle containing 30-50 ml Eagle's minimal essential medium with 10 mmol/l N-2-hydroxyethylpiperazine-N1-2-ethanesulphonic acid (HEPES) and 20% fetal calf serum. Reducing agents, which have been shown to be toxic to tissue cells, were not used in these experiments. The treponemes were extracted from the sliced syphilitic rabbit testes by shaking at 34°C for approximately 30 minutes. The T pallidum suspension was centrifuged at 1000 $x$ g for 10 minutes to remove rabbit tissue cells. The supernatant was recentrifuged at 8000 $x$ g for 30 minutes to separate the treponemes, which were then resuspended to the required concentration (10$^5$/ml) for injection into rabbit testicles.

Isolation and maintenance of tissue culture cells
Primary cultures of fibroblastic cells were obtained from one day old New Zealand baby rabbit genital organs (BRGO). The testis tissue was cut, minced, and stirred in phosphate buffered saline (PBS) containing 0.025% trypsin (Sigma), and 100 U/ml penicillin and 100 μg/ml streptomycin (Glaxo) at 37°C for 30 minutes. The cells were collected, centrifuged at 1000 $x$ g for 10 minutes, and resuspended in Eagle's minimal essential medium supplemented with 20% fetal calf serum, 10 mmol/l HEPES, and antibiotics as above. The cells were then grown in 10% fetal calf serum without antibiotics at 37°C, and the medium was changed every three days. The confluent monolayers were subcultured using PBS containing 0.025% trypsin and 0.001% ethylenediamine tetraacetate acid (Sigma) to remove cells from the glass. Carbon dioxide and bicarbonate were not used for maintenance of BRGO cells.
COINCUBATION OF BRGO CELLS WITH
\(^{3}H\)-THYMIDINE AND SYPHILITIC RABBIT SERUM
About \(10^{3}\) BRGO cells were seeded into each well of a
96 well microtitre plate (Sterilin, England) and allowed to
attach for 12 hours. This was followed by washing
with serum free medium and adding 200 \(\mu\)l syphilitic
rabbit serum (20% final concentration). Serum from
rabbits injected with heat killed \(T\) pallidum was
added to control wells. After aerobic coincubation
for 24 hours, 0·5 \(\mu\)Ci \(^{3}H\)-thymidine (20 Ci/mmol,
Amersham International, Amersham, England) was
added to each well. After incubation for a further 24
hours the BRGO cells were made soluble with 150 \(\mu\)l
lysis buffer (0·5 mol/l sodium hydroxide and 0·5%
sodium dodecyl sulphate) and washed sequentially
with 5% trichloroacetic acid, water, and methanol. The
trichloroacetic acid precipitates were collected on
to glass fibre filters (Flow Laboratories, Melbourne,
Australia) and their radioactivity measured using a
Packard Tri-Carb \(\beta\) scintillation counter, with
2,5-diphenyloxazole 5 g/l and 1,4-bis [2-(3-methyl-5-
phenyl-oxazolyl)]-benzene 0·4 g/l in toluene as the
scintillation fluid.

Results

EFFECT OF SYPHILITIC RABBIT SERUM ON
DNA SYNTHESIS IN BRGO CELLS
Serum from rabbits injected with heat killed \(T\) pallidum (50 \(\times\) \(10^{5}\)/testis) had no inhibitory effect
on DNA synthesis in BRGO cells, while serum taken
from rabbits during early syphilis (6 to 30 days after
infection) did inhibit DNA synthesis. The figure
shows that synthesis was only 40-70% of that using
day 0 rabbit serum from the same rabbits. Serum
taken later in the disease (40 to 120 days after
infection), however, did not appreciably inhibit the
BRGO cells. Heat inactivated and non-inactivated
serum samples showed similar results, indicating that
the toxic factor in the syphilitic rabbit serum was
stable to 56°C for 30 minutes (data not shown).

Serum taken from eight other rabbits during peak
syphilitic orchitis (11 to 14 days after infection) all
inhibited DNA synthesis in BRGO cells by between
64% and 93% (mean 81%), compared with serum from
rabbits injected with heat killed or sonicated \(T\) pallidum (data not shown).

Discussion

At the peak of syphilitic orchitis a soluble factor,
which is cytostatic for rabbit cells in culture and
apparently of treponemal origin, is present in rabbit
testes infected with \(T\) pallidum. This toxic substance
may play a part in destruction of local tissue in early
syphilis. We have now detected a toxic substance in
the serum of syphilitic rabbits up to 30 days after
infection. This toxin may reasonably be assumed to
be of the same origin as the cytotoxins in whole \(T\)
pallidum organisms and syphilitic rabbit testes. It
may be responsible for some of the systemic effects
of disseminated (secondary) syphilis. The effect of
this toxic factor on cells of the host’s immune system
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has not yet been tested, but the transient immunosuppression seen in experimental and human syphilis may well be mediated by such a mechanism.6-8

Apart from inhibition of DNA synthesis, morphological changes and detachment of BRGO cells were seen after three days' exposure to 10^8 or more treponemes/ml.1 Uninfected BRGO cells were normal in appearance. The relatively low growth ceiling for T pallidum seen in tissue culture9 may be due to inactivation of the mammalian cells by a treponemal toxic factor.

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