SHORT ARTICLE

ANTIMICROBIAL EFFECTS OF A MACROPHAGE-DERIVED CYTOTOXIN FROM THE SERUM OF BCG-PRIMED RABBITS (TUMOUR NECROSIS SERUM)

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SUMMARY. Previous studies have shown that a macrophage-derived cytotoxin, found in high titre in the serum of BCG-primed rabbits, killed malarial parasites and certain tumour cells. In this study, the macrophage cytotoxin was tested for activity against microorganisms other than protozoa. No activity was seen against a range of gram-negative or gram-positive bacteria or against *Candida albicans*. It is concluded that the macrophage cytotoxin has limited anti-microbial activity, perhaps restricted to certain intra-cellular parasites.

INTRODUCTION

Immunisation with *Bacillus Calmette Guérin* (BCG) or *Propionibacterium acnes* (formerly *Corynebacterium parvum*) can protect against subsequent challenge with antigenically unrelated organisms that include protozoa (Clark, Allison and Cox, 1976; Ortiz-Ortiz, Gonzalez-Mendoza and Lamoyi, 1975), fungi (Sher *et al.*, 1975), bacteria (Adlam, Broughton and Scott, 1972; Baughn, Musher and Knox, 1977) and viruses (Cerutti, 1974), as well as certain transplanted tumours (Halpern *et al.*, 1966; Woodruff and Boak, 1966). This cross resistance is usually considered to be due to activation of the phagocytic and oxidative capacities of the macrophage. However, we noted recently that a macrophage-derived cytotoxin isolated from the serum of BCG- or *P. acnes*-primed animals (tumour necrosis serum), killed both malarial parasites and tumour cells (Playfair, Taverne and Matthews, *in press*). This has prompted us to test whether the macrophage cytotoxin can kill microorganisms other than protozoa. The test organisms have been chosen because BCG or *P. acnes* has been shown to induce cross-resistance against them or closely related organisms.

MATERIALS AND METHODS

Tumour necrosis serum. A New Zealand White rabbit (2.25 kg) was given one vial of Glaxo percutaneous BCG (50–250 viable organisms/ml of saline) by intravenous injection, followed 14 days later by an intravenous injection of 100 µg of endotoxin (Lipopolysaccharide B from *Escherichia coli* O55:B5, Difco). The rabbit was bled immediately before the endotoxin was given to obtain the control serum and 2 h after the endotoxin injection to obtain the tumour necrosis serum. When assayed against the mouse L929 tumour cell line (Matthews, 1982), the tumour necrosis serum had a cytotoxin titre of $> 10^5$ compared with $< 10^2$ for the control serum.

Test microorganisms. Eleven test strains of nine species of bacteria and one strain of *Candida albicans* were studied (table). All were obtained from the bacteriology service of the University Hospital of Wales except for the *Corynebacterium hofmani* strains which were kindly provided by Dr I. Zamiri (Diphtheria Reference Laboratory, Cardiff). They were grown aerobically for 24 h on blood-agar plates and a stock suspension of each test organism was made in isotonic saline to give an optical density of 0.10 at 620 nm.

Pour-plate assay for microbial growth inhibition. Three-ml volumes of Bacto Antibiotic

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TABLE

Comparison of the effects of saline, control serum and tumour necrosis serum on colony formation by *C. albicans* and nine species of bacteria

Test microorganism	Mean colony count ($\pm$ SD)/dish in the presence of		
	Saline	Control serum	Tumour necrosis serum
<i>Candida albicans</i>	211 $\pm$ 10	210 $\pm$ 7	216 $\pm$ 10
<i>Escherichia coli</i>	98 $\pm$ 9	106 $\pm$ 4	101 $\pm$ 4
<i>Salmonella</i> sp.	194 $\pm$ 4	195 $\pm$ 3	198 $\pm$ 6
<i>Salmonella agama</i>	58 $\pm$ 3	26 $\pm$ 6	48 $\pm$ 11
<i>Shigella sonnei</i>	207 $\pm$ 6	168 $\pm$ 8	220 $\pm$ 7
<i>Pseudomonas aeruginosa</i>	292 $\pm$ 14	328 $\pm$ 6	328 $\pm$ 13
<i>Listeria monocytogenes</i>	459 $\pm$ 15	507 $\pm$ 34	468 $\pm$ 21
<i>Staphylococcus aureus</i>	1088 $\pm$ 43	1042 $\pm$ 56	1065 $\pm$ 37
<i>Streptococcus faecalis</i>	274 $\pm$ 10	281 $\pm$ 16	280 $\pm$ 4
<i>Corynebacterium hofmani</i>	205 $\pm$ 21	232 $\pm$ 7	304 $\pm$ 19
<i>C. hofmani</i> 375	201 $\pm$ 14	222 $\pm$ 19	209 $\pm$ 21
<i>C. hofmani</i> 300	310 $\pm$ 33	862 $\pm$ 12	916 $\pm$ 45

medium 1 (Difco) were melted at 100°C and cooled to 56°C before 300 μ l of horse serum (Difco) were added. After mixing gently by inversion, the medium was poured into 6-cm plastic petri dishes and immediately 50–200- μ l volumes of saline, control serum or tumour necrosis serum were added with 100 μ l of the microbial suspension (a 1 in 25 000 or 1 in 100 000 dilution of the stock). The plates were rotated to disperse the organisms and then allowed to set. Colonies were counted after overnight incubation at 37°C. Each test was done in triplicate. The ratio of volume of tumour necrosis serum to number of organisms was of the same order as that found to be effective against plasmodia.

RESULTS AND DISCUSSION

Because tumour necrosis serum has high titres of mediators such as interleukin 1 and interferons as well as macrophage cytotoxin, any antimicrobial effect seen with the whole serum could not be directly assigned to the cytotoxin. However, the macrophage cytotoxin has reduced stability after purification; therefore, we decided to use crude serum in the initial experiments with the intention of testing purer cytotoxin preparations if any effects were seen with whole serum. The microorganisms chosen for study were those considered most likely to be susceptible on the basis of published reports on the protection afforded against them by BCG or *P. acnes*.

In the presence of tumour necrosis serum, there was no reduction in the numbers of colonies of any of the microorganisms tested (table), nor was there an apparent reduction in colony size.

Carswell *et al.* (1975) noted that the serum of animals challenged with endotoxin 2 weeks after primary intravenous injection of BCG or *P. acnes* caused necrosis of certain transplanted tumours and was cytotoxic to some tumour cell lines *in vitro*. Because of the relative sensitivity of the in-vitro assays, most studies on the chemistry and biology of the anti-tumour effect have relied upon in-vitro assays. Thus, in the rabbit, the cytotoxin has been shown to be a protein of mol. wt 39 000–55 000 and with an isoelectric point at pH 5.1–5.2 (Matthews, Ryley and Neale, 1980; Ruff and Gifford, 1980); it is produced by macrophages (Matthews, 1981; Fisch and Gifford, 1983). Although it was originally thought that the same factor (tumour necrosis factor) was responsible for both in-vivo and in-vitro anti-tumour effects (Green *et al.*, 1976) more recent studies suggest otherwise (Kull and Cuatrecasas, 1981). It is clear, however, that it is the macrophage-derived cytotoxin detected by in-vitro assays that kills malarial parasites (Playfair *et al.*, in press).

The macrophage cytotoxin had no effect on any of the organisms tested in the present study. These organisms differ from the cytotoxin-sensitive plasmodia in that they were tested whilst growing extracellularly. This suggests that the cytotoxin may be effective only against certain intracellular infections, presumably because it recognises a cell-surface alteration common to both plasmodial infection and malignant transformation. There is a superficial similarity between the cytotoxin and the interferons in terms of activity against both intracellular infections and tumour cells. However, the macrophage cytotoxin has minimal antiviral activity and can be distinguished from the interferons by physicochemical criteria (Matthews, 1979).

In conclusion, this study suggests that the macrophage cytotoxin has limited antimicrobial activity, possibly restricted to certain intracellular parasites.

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