

RESEARCH NOTE

TUMOUR REGRESSION IN MICE AFTER TREATMENT WITH EXTRACTS OF THE BONE MARROW FROM CATTLE HARBOURING TICK-BORNE INFECTIONS

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ABSTRACT

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Complete regression of a transplantable fibrosarcoma of mice was induced in an average of 86% of animals receiving repeated subcutaneous injections of an extract of the bone marrow from cattle harbouring *Babesia* parasites. Extracts from cattle harbouring *Theileria mutans* produced regression of tumours in an average of 63% of the recipients. Splenic extracts from the same animals and bone marrow extracts from an anaplasmosis carrier were less effective. Bone marrow extracts from control animals known to be free from these infections did not enhance the survival of tumour-bearing mice.

Résumé

RÉGRESSION DE TUMEURS CHEZ LA SOURIS APRÈS TRAITEMENT PAR DES EXTRAITS DE MOELLE OSSEUSE DE BOVINS HÉBERGEANT DES INFECTIONS TRANSMISES PAR LES TIQUES

On a obtenu la régression complète d'un fibrosarcome transplantable de la souris chez 86% en moyenne des animaux qui avaient reçu des injections sous-cutanées répétées d'un extrait de moelle osseuse prélevé sur des bovins infectés par de *Babesia*. Des extraits pris aux bovins abritant *Theileria mutans* ont amené une régression des tumeurs chez 63% en moyenne des animaux récepteurs. Des extraits de rate des mêmes donneurs et des extraits de moelle osseuse d'animaux-contrôle exempts de ces infections n'ont pas amélioré la survie des souris porteuses de tumeurs.

Extracts of spleen, bone marrow and lymph nodes from apparently healthy cattle were used by Fichera (1934) in the treatment of human neoplasms. One hundred of the 300 patients he treated had inoperable cancer. At the end of 3 years complete regression had occurred in 9% of the patients and 8% of the tumours had become stationary. In a previous study, King (1966) found that the treatment of various malignant neoplasms in both human and animal patients with an extract of bone marrow obtained from cattle infested with cysticercosis was remarkably effective.

The growth of both allogeneic and syngeneic tumours has been shown to be markedly inhibited in rats and mice infested with *Nippostrongylus brasiliensis* and *Schistosoma mansoni* (Keller, Ogilvie & Simpson, 1971). Keller *et al.* (1971) also demonstrated that inhibition of tumour growth is effected by macrophages non-specifically activated by IgE which is synthesized in response to the helminth infestation (Capron, Dessaint, Capron & Bazin, 1975).

Macrophages are also activated by various other non-specific factors, including intracellular parasites such as *Toxoplasma* or *Besnoitia* (Hibbs, Lambert & Remington, 1972) and they have been shown to play an important part in the cytostatic elimination of tumours (Keller, 1973; Krahenbuhl & Remington, 1974). It was therefore decided to test bone marrow extracts from cattle infected with intracellular protozoal parasites for cytostatic activity against neoplasms.

In order to obtain controlled results, a transplantable tumour of mice was selected for this purpose. The M(52)B cell line, which was originally established from a murine sarcoma virus-induced fibrosarcoma, was maintained *in vitro*, using standard cell-culture techniques. Subcutaneous injection of new-born allogeneic outbred mice with 5×10^6 cells produced tumours of about 4 mm in diameter within 14 days. Treatment, which commenced when the mice were

10-14 days old, consisted of daily subcutaneous injections of 0,1 ml of extract for the first 3 days, followed by daily doses of 0,2 ml. Controls consisted of 5 litters of mice which were left untreated. The size of each tumour was plotted weekly on graph paper.

Bone marrow and spleen samples were obtained from cattle either naturally or experimentally infected with various tick-born organisms.

In one series of experiments bone marrow extracts were used from cattle originating from two farms in the Zoutpansberg district of Transvaal and showing *Theileria mutans* in their blood smears. In a second series the animals concerned were preimmunized in the laboratory against *Babesia bovis* and *Babesia bigemina*. An *Anaplasma centrale* preimmunized animal provided material for another experiment. Normal bone marrow and spleen were obtained from cattle born and reared under tick-free conditions at this Institute.

Extracts were made by homogenizing equal parts of tissue and 0,1 M phosphate buffer (pH 7,2) in a Braun homogenizer. After being allowed to stand for 40 min, the suspension was filtered through Whatman No. 1 filter paper and clarified by centrifugation at 2 000 r.p.m. for 40 min. The supernatant was then sterilized by filtration through a Seitz filter, freeze-dried in 4 ml volumes and stored at 4 °C. Each vial was reconstituted for use with 4 ml of sterile distilled water. Filtrate was also stored frozen at -20 °C.

The results of 12 experiments are summarized in Table 1.

Four only of 42 untreated control mice survived with complete regression of tumours, a spontaneous survival rate of 9,5%. The other mice died within 3-4 weeks with tumours often approaching the size of the animal. Treatment with either bone marrow or spleen extract from uninfected control animals gave comparable results, that is, survival rates of 8,0% and 12,5% respectively.

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TABLE 1 Survival rate of tumour-bearing mice treated with various extracts

Bovine donors		Mouse recipients		
Parasitic infection	Extract	Number treated	Number survived	Survival rate %
Untreated control.....	—	42	4	9,5
Negative control.....	Bone marrow.....	37	3	8
Negative control.....	Spleen.....	24	3	12,5
<i>Babesia</i>	Bone marrow.....	22	20	91
<i>Babesia</i>	Bone marrow.....	21	17	81
<i>Babesia</i>	Bone marrow 5 × concentrated.....	28	12	43
<i>Babesia</i>	Bone marrow + Spleen (50:50).....	27	14	52
<i>Babesia</i>	Spleen.....	31	11	35
<i>Babesia</i>	Spleen.....	29	11	38
<i>Theileria mutans</i>	Bone marrow.....	50	29	58
<i>Theileria mutans</i>	Bone marrow.....	32	22	69
<i>Anaplasma centrale</i>	Bone marrow.....	32	6	19

In contrast, the highest survival rates of up to 91% were obtained with bone marrow extract obtained from the *Babesia*-infected cattle. Spleen extract from these animals was less effective, resulting in a survival rate of only about 36% and a mixture of spleen and bone marrow extracts gave intermediate results. Concentration of the bone marrow extract had the effect of diminishing its effectivity, probably because of the loss of the active principle. Bone marrow extracts from animals harbouring *Theileria mutans* were also reasonably effective, producing complete tumour regression in about 63% of animals, but those obtained from an anaplasmosis carrier had a low activity and a survival rate of only 19%.

The tumours in the treated mice had shrunk within days of the commencement of the treatment and had completely disappeared within 1-2 weeks. If a tumour continued to increase in size, it was a sign that regression would not take place. Nevertheless, the tumours in the treated mice that failed to show regression often grew at a slower rate than in the untreated controls and the animals usually survived 4-5 weeks.

These results indicate that a soluble factor is present in the bone marrow of cattle harbouring intracellular protozoan and other parasites, which can induce a non-specific cytotoxic reaction against tumour cells in mice. *Babesia* seems to be more effective in inducing the production of this factor than *Theileria*, and *Theileria* is considerably more effective than *Anaplasma*. The nature of this factor and the mechanism of the cytotoxic reaction have not been investigated. It is, conceivable, however, that an immunoglobulin activating the recipient's macro-

phages could be involved, in accordance with the mechanisms proposed by Keller & Hess (1972), Hibbs *et al.* (1972) and Capron *et al.* (1975).

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