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Special Article

Hyporesponsiveness of Macrophages from C_3H/HeJ (Endotoxin-resistant) Mice to the Antitumour Agent Flavone Acetic Acid (NSC 347512)

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THE mode of action of flavone acetic acid (FAA), a new antitumour agent currently undergoing clinical trial [1], contrasts in several ways with that of conventional cytotoxic agents, with several studies implicating the involvement of host cytotoxic mechanisms in mediating its antitumour effects [2, 3]. The tumouricidal activity of macrophages can be enhanced by FAA *in vitro*, indicating that the macrophage may constitute the target host cell that interacts directly with FAA [4]. Since C_3H/HeJ mice have defective macrophage responses [5–13], we have tested the ability of macrophages from C_3H/HeJ mice to respond to FAA *in vitro* and the capacity of FAA to induce haemorrhagic necrosis of tumours implanted in C_3H/HeJ recipients. In this report, we demonstrate that C_3H/HeJ macrophages are defective in their *in vitro* response to FAA, but that mammary M16C tumours or transplanted spontaneous tumours growing s.c. in C_3H/HeJ or C_3H/HeN mice nevertheless undergo haemorrhagic necrosis 24 h after *in vivo* treatment with FAA (500 mg/kg).

FAA was from the National Cancer Institute, U.S.A. and lipopolysaccharide was from Sigma, U.S.A. αMEM (Gibco) was supplemented with foetal bovine serum (10%, Gibco NZ Ltd), 2-mercaptoethanol (50 μM) and antibiotics. C_3H/HeJ (stock from Jackson Laboratories, U.S.A.) and C_3H/HeN (stock from National Institutes of Health and Sciences, U.S.A.) mice were bred in the laboratory animal facilities under constant temperature and humidity with sterile bedding and food. The M16C mammary carcinoma was provided by Dr W.R. Leopold, Parke-Davis Division, Warner-Lambert, U.S.A. Two adenocarcinomas which had developed spontaneously in C_3H/HeN mice were passaged s.c. in C_3H/HeN mice before use. Tumours were grown to a diameter of approx. 10 mm in carrier mice, removed surgically and cut into 1 mm^3 fragments and implanted in anaesthetized recipient mice.

Peritoneal exudate (PE) cells were collected from mice and viable white cells were counted by eosin exclusion. PE cells were plated in 96 well V-bottom microtitre trays (Linbro) in the presence of ^{51}Cr-labelled tumour targets (5×10^3 cells per well) in a total volume of 0.2 ml culture medium. The tumour target used was the P815 mastocytoma (provided by Dr J. Marbrook), maintained in culture and labelled by incubation for 45 min at 37°C with 200 μCi sodium ^{51}Cr-chromate in saline and washing three times. The amount of radioactivity released following incubation for 18 h at 37°C in 5% CO_2 was measured by removing 0.1 ml of supernatant and counting in a gamma counter (LKB Wallac 1270 Rackgamma 11, Wallac, Finland). Lytic activity in this assay is mediated by Thy-1 negative, glass adherent dexamethasone inhibitable cells in the PE population [4].

PE cells from C_3H/HeN and C_3H/HeJ mice were incubated with varying concentrations of FAA (dissolved to 30 mg/ml in 5% sodium bicarbonate and diluted in culture medium) to determine their ability to lyse P815 targets at an effector:target (E:T) ratio of 100:1 (Fig. 1). The response by C_3H/HeN PE cells was similar to that reported previously for PE cells from ($C_{57}BL/6 \times DBA/2$) and $C_{57}BL/10$ mice

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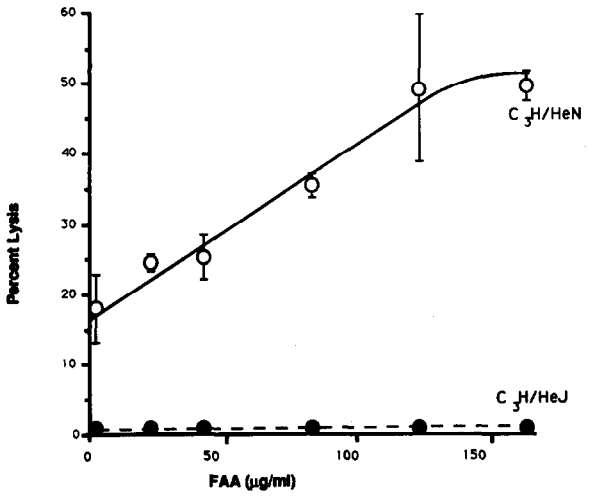

Fig. 1. Response to FAA by PE cells from C_3H/HeN and C_3H/HeJ mice. PE cells were incubated with FAA and ^{51}Cr-labelled P815 targets at 100:1 E:T ratio. Lysis was measured after 18 h.

[4] with lytic activity increasing with increasing concentrations of FAA. In contrast, no response was detectable in cultures of C_3H/HeJ PE cells at all FAA concentrations tested, nor was activity detectable in the absence of PE cells with FAA doses up to 160 µg/ml. A control study used LPS (0.1–20 µg/ml; dissolved in growth medium) to activate macrophages. Tumouricidal activity of C_3H/HeN PE cells against P815 targets at an E:T ratio of 100:1 was lower than that found with FAA (typically 15% lysis) with no clear relationship between dose of LPS and the response in the cultures. No activity was detectable in cultures of C_3H/HeJ PE cells stimulated with LPS.

The effect of different E:T ratios on the activity of PE cells incubated with and without FAA is shown in Fig. 2. C_2H/HeN PE cells cultured with FAA (150 µg/ml) showed increasing lytic activity

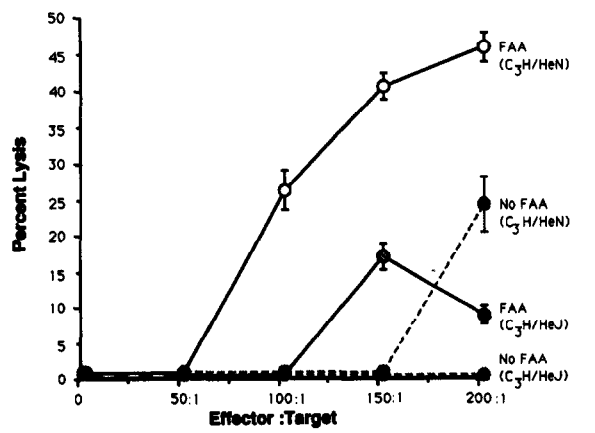

Fig. 2. Activity by PE cells from C_3H/HeN and C_3H/HeJ at different E:T ratios with or without FAA. Varying numbers of PE cells were incubated 18 h with ^{51}Cr-labelled P815 targets either with or without 150 µg/ml FAA.

with increasing spontaneous activity against P815 targets only at E:T ratios of 200:1 (Fig. 2). The level of spontaneous activity from C_3H/HeN PE cells at a given E:T ratio tended to vary from experiment to experiment but was always significantly lower than the activity detected in FAA-stimulated cultures at the same E:T ratio. No spontaneous activity was present in C_3H/HeJ cultures at any E:T ratio tested. In the presence of FAA at 150:1 E:T ratio some activity was detectable but this was much lower than that found with C_3H/HeN cultures and was not sustained at higher E:T ratios (Fig. 2). The results in Figs. 1 and 2 are representative of a number of experiments which show that the tumouricidal response to FAA by PE cells from C_3H/HeN and C_3H/HeJ mice is clearly different.

Our results showing that C_3H/HeJ macrophages are hyporesponsive to FAA *in vitro* lend support to the hypothesis that a generalized defect is present in C_3H/HeJ macrophages rather than insensitivity to LPS alone [11–13]. LPS resistance has been traced to a single co-dominantly inherited, autosomal gene [5–7] and we have yet to determine whether the defective response to FAA in C_3H/HeJ mice is also controlled by the lps^d gene.

Since C_3H/HeJ macrophages exhibited a defective response to FAA *in vitro* (Figs. 1 and 2), we examined whether the antitumour effects of FAA were impaired in C_3H/HeJ mice. The M16C mammary carcinoma, as well as two adenocarcinomas which had spontaneously arisen in C_3H/HeN mice, were implanted s.c. into either C_3H/HeJ or C_3H/HeN mice. All three tumours grew equally well in both strains of mice. Tumour-bearing mice were injected i.p. with FAA (500 mg/kg) and after 24 h tumours were removed for histological assessment of necrosis as previously described [14]. The M16C and both spontaneous adenocarcinomas were found to be sensitive to FAA. Greater than 80% necrosis of all three tumours was obtained in both C_3H/HeJ and C_3H/HeN mice.

These results could be interpreted to imply that macrophages are not involved in the induction of haemorrhagic necrosis by FAA. However, the defects in C_3H/HeJ macrophage functions can be corrected using lymphokines [13] and interferons [15]. Furthermore, FAA treatment *in vivo* has been shown to induce the production of interferons α/β [16] which could *in vivo* reverse the hyporesponsiveness of C_3H/HeJ macrophages to FAA. We conclude that C_3H/HeJ macrophages are hyporesponsive to FAA *in vitro*, but that the ability of FAA to induce haemorrhagic necrosis of tumours *in vivo* in C_3H/HeJ mice is not impaired. Further work is required to determine the involvement of macrophages in the response of tumours to FAA.

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