Adjuvant Induced Resistance to Tumor Development in Mice[1] (36296)

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Mice chronically infected with intracellular protozoa were recently shown to have increased resistance to autochthonous (1–3) and transplanted tumors (1, 2) and activated peritoneal macrophages of these mice had the capacity to destroy L cells or tumor target cells *in vitro* (4). We have suggested that the many agents which have been used to nonspecifically increase host resistance to murine tumors [poly I:poly C, endotoxin, zymosan, *Corynebacterium parvum, Bordetella pertussis, Bacillus Calmette-Guerin* (BCG) or methanol insoluble fraction of BCG] may operate, in part at least, through the common mechanism of stimulating activation of host macrophages with increased cytotoxic capabilities (2). Since complete Freund's adjuvant (CFA) has been shown to increase nonspecifically resistance to *Listeria* in mice, and since the mechanism of this resistance appears to be CFA activation of macrophages (5), it was considered of interest to determine if CFA also protects against autochthonous and transplanted tumors.

Materials and Methods. Female Swiss–Webster (SW), DBA/2 and AKR mice were obtained from Simonsen Breeding Laboratory, Gilroy, CA; retired breeder C_3H/HeJ mice were purchased from Jackson Memorial Laboratories, Bar Harbor, ME. Mice used for evaluation of resistance to any given tumor were age and weight matched. Complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, MI) was mixed with equal volumes of Hanks' balanced salt solution (HBSS) and mice were injected with 0.1 ml of the resulting emulsion intraperitoneally (ip) and 0.1 ml subcutaneously. Incomplete Freund's adjuvant (ICFA) was prepared in the same manner and mice were inoculated with the same quantity and by identical routes as mice pretreated with CFA. Other mice received HBSS alone at the same injection sites. Tumors used, methods of transplantation, and evaluation of tumor growth have been described (2). The strain of *L. monocytogenes* and its preparation have been described (6).

Results. Challenge with the facultative intracellular bacteria, *Listeria monocytogenes*, was used to evaluate nonspecific resistance to an unrelated intracellular organism. Mice were pretreated with CFA, ICFA, or HBSS 10 weeks before *Listeria* challenge. All strains of mice pretreated with CFA showed significantly greater survival and prolonged time to death following ip inoculation of *Listeria* (Fig. 1a). CFA stimulated marked resistance to *Listeria* while ICFA and HBSS controls were not protected. On the same day that the SW mice (Fig. 1a) were challenged with *Listeria*, other mice from the same pretreatment groups were grafted with 1×10^6 Sarcoma 180 cells ip. Mice pretreated with CFA showed significantly greater survival (*e.g.*, at 28 days $p = <.001$) and delayed time to death when compared to ICFA and HBSS controls (Fig. 1b). Greater survival and prolonged survival was noted in SW mice following ip inoculation of 0.2 ml of a 10% suspension of Friend leukemia virus infected spleen cells 11 weeks after pretreatment with CFA (*e.g.*, at 2.7 months $p = < .001$) (Fig. 1c) and delayed time to death was noted in DBA/2 mice grafted with 1×10^5 leukemia

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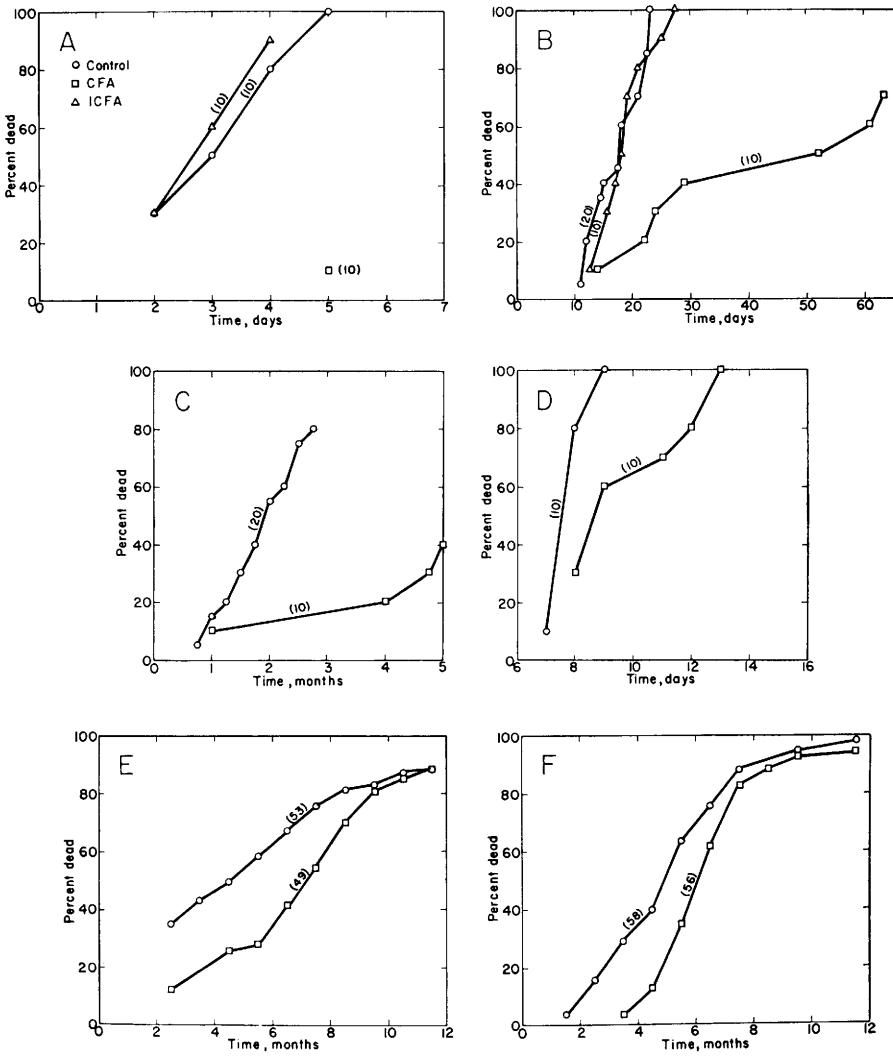

FIG. 1. Effect of CFA on resistance to *Listeria,* transplanted tumors and autochthonous tumors in mice; a = incidence of death after ip inoculation of 1.7×10^7 *Listeria* organisms in SW mice; b = incidence of death after ip inoculation of 1×10^6 Sarcoma 180 cells in SW mice; c = incidence of death after ip inoculation of a 10% suspension of Friend leukemia virus infected spleen cells in SW mice; d = incidence of death after ip inoculation of 1×10^5 leukemia ^{L}1210 cells in DBA/2 mice; e = incidence of palpable spontaneous mammary tumors in C_3H/HeJ mice; f = incidence of death due to spontaneous leukemia in AKR mice. In a, time 0 = day of *Listeria* challenge. In b, c, and d, time 0 = day of tumor transplant. In e, and f, time 0 = day of inoculation of CFA or HBSS. Figures in parentheses = number of mice.

^{L}1210 cells ip 9 weeks after pretreatment with CFA (*e.g.*, at 9 days $p = < .04$) (Fig. 1d).

CFA also stimulated increased host resistance to autochthonous tumors in mammary adenocarcinoma in C_3H/HeJ mice (*e.g.*, at 5.5 months $p = < .001$) (Fig. 1e), and thymic lymphoma in AKR mice (*e.g.*, at 4.5 months $p = < .001$) (Fig. 1f). The protective effect to these spontaneous tumors appeared to be inversely proportional to the time interval following administration of CFA. For example, palpable mammary tumors in C_3H/HeJ mice were initially sup-

pressed by CFA treatment, but 11 months following CFA administration tumor incidence was identical to that of HBSS controls.

Discussion. Complete Freund's adjuvant stimulates multiple host resistance factors, specific as well as nonspecific (7–12). Reports of the effect of CFA on tumor development are conflicting. Adenovirus type 12 oncogenesis was reduced in CFA treated hamsters (13) as was Friend leukemia virus induced splenomegaly in mice (14). Mice inoculated with Rauscher leukemia virus, following a series of weekly injections of CFA in combination with virus unrelated antigens, showed increased survival and delayed appearance of circulating nucleated erythrocytes characteristic of the leukemia (15). In a study designed to immunize virgin female C_3H mice with a mammary tumor homogenate in CFA before spontaneous mammary tumors had appeared, Isojima and coworkers noted the unexpected finding of remarkable reduction of mammary tumors in control mice which received CFA alone (16). Zbar and coworkers noted no inhibition of tumor growth when a transplantable syngenic hepatoma was inoculated without admixed mycobacteria antigens in inbred guinea pigs pretreated with CFA. However, by producing a delayed hypersensitivity reaction at the immediate site of the tumor graft and by insuring intimate contact between CFA sensitized cells, mycobacterium antigens, and the antigenically unrelated tumor cells, progressive growth of the tumor graft was partially or completely nonspecifically inhibited (17). On the other hand, CFA enhanced formation of tumors induced by Rous sarcoma virus in chicks (18), simian virus 40 in hamsters (19), polyoma virus in mice and Rous sarcoma virus in rats (20). CFA was also found to decrease host resistance to adenovirus type 12 transformed cells in hamsters (21).

These conflicting reports are difficult to explain, but probably depend on complex interactions involving many variables which include tumor type, species, sex, and age of animals used, timing of CFA administration, *etc.* In the present study, increased resistance to tumor development was noted in each of the experimental models. Treatment with CFA was before the development of overt autochthonous neoplasia, or 9 to 11 weeks prior to tumor transplantation, and it should be emphasized that in each case the protection was preventive in nature rather than therapeutic. If CFA inoculations had been repeated, providing ongoing high level stimulation, as was done by Isojima and his coworkers (16), a more impressive reduction of tumor development in the two autochthonous tumor models studied might have been noted. It appears the protective effect of CFA decreases gradually with time, since tumor development approached that of controls 9 months after CFA administration in the case of spontaneous mammary carcinoma in C_3H/HeJ mice and 6.5 months after CFA administration in the case of thymic lymphoma in AKR mice.

As was noted previously in mice chronically infected with intracellular protozoan parasites (2), mice pretreated with CFA have enhanced resistance to the facultative intracellular bacteria *L. monocytogenes* as well as to transplanted and autochthonous tumors. Peritoneal macrophages from mice with chronic intracellular protozoan infections are activated and have increased microbiocidal (22) and cytotoxic capabilities (4) *in vitro*. Mice pretreated with CFA also have a population of activated peritoneal macrophages with increased *in vitro* microbiocidal potential (5) and, as the results presented in the accompaning paper demonstrate, *in vitro* cytotoxic effect for tumor target cells but not for syngenic or allogenic mouse embryo cell strains (23).

In the two experimental models we have used to study nonspecific resistance to intracellular infection and neoplasia, mice chronically infected with intracellular parasites or pretreated with CFA, parallel results have been obtained. We believe mechanisms of host resistance to intracellular infectious agents and neoplasia are related in a fundamental way, and that the activated macrophage is a common effector arm for expression of this resistance. In addition, we believe the diverse agents capable of nonspecific stimulation of host resistance to neoplasia operate, in part at least, through the acti-

vated macrophage (2).

Summary. Mice pretreated with complete Freund's adjuvant had an enhanced resistance to autochthonous and transplanted tumors. Delayed time to death and/or increased survival was noted in CFA pretreated mice grafted intraperitoneally with Sarcoma 180, leukemia $^{L}1210$, or Friend leukemia spleen cells. In addition, CFA pretreatment caused a statistically significant delay in spontaneous mammary tumor development in C_3H/HeJ mice and spontaneous leukemia in AKR mice. We propose that host resistance to intracellular infectious agents and neoplasia is related in a fundamental way and the activated macrophage is a common effector arm for expression of this resistance. It is also suggested that a nonimmunologic growth control mechanism such as we have described offers a rapid acting homeostatic process for destruction of cells with abnormal growth properties *in vivo*.

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