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Effect of Microbial Antigens on Irradiation Mortality in Mice.* (25290)

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Boivin-type endotoxins from *Proteus morganii* decrease mortality in x-irradiated mice (1,2). It is also known that endotoxins from *Salmonella typhosa, Escherichia coli,* and *Serratia marcescens* increase the survival of irradiated mice, rats, and hamsters(3,4). Since the chemically isolated lipopolysaccharide substances called endotoxins or toxic somatic antigens are thermostable antigenic components associated with the bacterial surface, bacterial vaccines and boiled culture supernatants, both of which would be expected to contain these somatic antigens, should also be effective in decreasing irradiation mortality. For this reason, the present preliminary investigation was initiated. We have found that mortality is definitely decreased by injection of Gram-negative antigens. Gram-positive antigens, however, appear ineffective in this respect. Other attempts to decrease mortality by means of bacterial antigens other than purified endotoxins have been unsuccessful (5).

Materials and methods. Thermostable antigens were prepared from *Klebsiella pneumoniae, Proteus morganii, Escherichia coli,* and an α hemolytic Streptococcus by heating saline suspensions of each organism for 1 hr in a boiling water bath. Following clarification, toxicity tests were performed on each of the sterile antigen solutions using non-irradiated mice. Groups of mice were injected intravenously with 0.1 ml of a sublethal dilution of each antigen 24 hr before irradiation. The following commercial (Parke, Davis) antigens were used: Streptococcus (Bio. 327), each ml containing saline extractable substances from 2×10^8 hemolytic and non-hemolytic Streptococci; Staphylococcus (Bio. 385), each ml containing soluble antigens from 1×10^9 *Staphylococcus aureus* and *Staphylococcus albus* mixed with Staphylococcus toxoid; Typhoid-Paratyphoid vaccine (Bio. 446), each ml containing 1×10^9 killed *Sal. typhosa,* 250×10^6 *Sal. paratyphi,* and 250×10^6 *Sal. Schottmulleri.* All of these antigens were used undiluted and given intravenously in a volume of 0.1 ml. A concentrated saline suspension of zymosan was heated in a boiling water bath for 1 hr, diluted as required with buffered saline (pH 7.0), and injected intravenously in a volume of 0.2 ml. Zymosan was obtained from Immunological Specialties, Los Angeles. Total body x-irradiation was delivered from a 200 kv Picker-Waite X-Ray therapy machine operated at 20 ma and a distance of 20 cm. Added filtration consisted of 0.5 mm Cu and 1 mm Al. Mice used were

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TABLE I. Survival of Mice Treated with Antigen 24 Hr before Irradiation with 700 r.

Antigen	30 day mortality	%	Level of significance of mortality reduction
Klebsiella (1:10)	17/30*	56	$P < .001$
Proteus (1:100)	14/30	46	"
Escherichia "	9/18	50	"
α Streptococcus	28/30	93	$P > .05$
Zymosan 300 γ	15/18	83	"
" 1000 γ	16/30	53	$P < .001$
Streptococcus	18/18	100	
Staphylococcus	18/18	"	
TAB	1/18	5.5	"
Controls	26/26	100	

* No. dead/total irradiated.

BUB females, 60-90 days old weighing approximately 25-30 g. Animals were kept in temperature regulated rooms and given tap water and Purina Lab Chow *ad libitum*.

Results. Irradiation mortality is decreased from 100% to approximately 50% in animals injected with heated Klebsiella, Proteus, or Escherichia antigens 24 hr before irradiation with 700 r (Table I). The interval of 24 hr between injection and irradiation was chosen for antigen screening since this interval had proven most effective with Boivin-type endotoxins from *Proteus morganii*. The best protection at 700 r is afforded by Typhoid-Paratyphoid vaccine (TAB), mortaltiy being reduced by 94% by a single injection 24 hr before irradiation.

Mortality is not significantly reduced, however, among animals injected with antigens from Gram-positive organisms. A dose of 300 γ of zymosan also fails to decrease mortality, but 1000 γ decreases mortality to approximately the same extent as do heated bacterial antigens. Since TAB vaccine proved most effective at 700 r, it was tested at 600 r, an irradiation dose at which post-irradiation treatment with endotoxin is effective (Table II). No deaths are observed among animals given a single injection 24 hr before irradiation. Post-irraditiaon treatment is less effective, but mortality is decreased by more than 64%. Multiple injections of TAB vaccine, given 7, 4, and 1 day before 600 r, significantly decrease mortality but are less effective than a single pre-irradiation injection ($P < .001$).

Long-term survival was recorded among TAB-treated mice irradiated with 600 r. In one experiment involving 43 such animals (alive at 30 days) 11 died within the succeeding 188 days (27%). In another experiment, 67 TAB-treated animals were alive at 30 days and 11 of these died in the succeeding 117 days (16%). A total of 11 non-treated irradiation controls were available for study (controls from both experiments) and in this group no deaths have been observed in the ensuing periods.

Discussion. Thermostable antigens from Klebsiella, Proteus, or Escherichia decrease irradiation mortality in mice. The active component is undoubtedly the endotoxin or somatic antigens from the respective organisms since these effects can be produced using Boivin-type endotoxins or other so-called purified somatic antigens or endotoxins(1-4). The extent of mortality reduction following treatment with heated Gram-negative antigens is less than that observed using TAB vaccine or that previously observed using Boivin-type endotoxin. This may, however, be a result of antigen dosage rather than a differential effectiveness of certain antigens, since higher doses of endotoxin appear less effective than lower doses(4). These doses of heated Gram-negative antigens were rather high since several injected animals died within 4 days, probably from the lethal effects of the endotoxins.

TAB vaccine is highly effective in reducing irradiation mortality and appears as effective as Boivin-type endotoxin in this respect. In fact, at 24 hr after irradiation with 600 r, injection of TAB vaccine results in a greater reduction of mortality (69%) than does an injection of *Proteus morganii* endotoxin (29%). Rowen *et al.*, studying the effects of

TABLE II. Increased Survival of Irradiated Mice (600 r) Treated with Typhoid-Paratyphoid Vaccine.

Time of inj.	30 day mortality	%	Level of significance of mortality reduction
7, 4, 1 day before irrad.	7/24*	29	$P < .02$
24 hr " "	0/36	0	$P < .001$
2 hr after "	8/36	22	"
24 hr " "	7/36	19	"
Controls	19/30	63	

* No. dead/total irradiated.

bacterial antigens on irradiation mortality, found mortality to be increased among mice injected with typhoid vaccine 5 days before irradiation(5). Although we have not tested the efficacy of TAB vaccine at this interval before irradiation, we previously found *Proteus morganii* endotoxin to be ineffective if given 4 days before irradiation. Rowen's negative results are probably a function of time of injection.

The finding reported here that multiple injections of TAB vaccine decrease mortality is at variance with observations of Smith *et al.* who reported no increased survival of mice given multiple injections of typhoid endotoxin(4). These differing results may be explained by the use of different antigen dosages. It appears, however, that mice become somewhat refractory to the protective effects of TAB vaccine or endotoxin following multiple injections. An interesting observation, based, however, on rather small numbers, is that after 30 days no deaths occurred among irradiation control animals, whereas considerable mortality was observed during this period among TAB treated animals. The protection afforded by TAB vaccine appears to be only temporary.

Although conflicting reports are present in the literature concerning the effect of zymosan on irradiation mortality(6,7), protective effects have recently been reported(8,9), and the results reported here confirm these positive results. Zymosan and somatic antigens or endotoxins have many properties in common. Both have a high polysaccharide content(10, 11), both induce tumor necrosis(12,13), both have an adjuvant effect on antibody production(14,15), and appropriate doses of both elevate properden titers(16). Although substances from some Gram-positive organisms have certain endotoxic properties(17), soluble antigens from Staphylococci and Streptococci are ineffective in decreasing irradiation mortality.

Summary. Mortality is reduced by approximately 50% in mice injected with zymosan (1000 γ) or with thermostable antigens from *Proteus morganii, Klebsiella pneumoniae,* or *Escherichia coli.* Soluble antigens from Gram-positive organisms, however, are ineffective. The greatest reduction of mortality is obtained by means of a Typhoid-Paratyphoid vaccine.

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