Ascites Production in 17 Mouse Strains. (26566)

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Ascitic fluid from immunized mice as a source of high titer antibody has been reported(1-4). Interest in ascites production arises also from the fact that with the BALB/cAnN mouse, ascites may be associated with development of plasma cell tumor(5).

Success in eliciting ascites through use of incomplete Freund's adjuvant has not been uniform in this laboratory. Judging from numerous personal communications from other investigators, difficulties have also been encountered elsewhere. The present report is concerned with factors affecting the reproducibility of the phenomenon of ascites production.

The principal factor reported here is that of mouse strain variation in ascitic fluid production and, in some instances, the effect thereon of including heat-killed *Staphylococcus aureus* in the ascites-inducing dose. Variation in the time course of production of ascitic fluid and the lethality of treatment are also observed . For the BALB/cAnN mouse, the occurrence of plasma cell tumors is noted.

Materials and methods. Procedures employed in the various experiments covered in this report have been described(4). Female mice of 17 different strains, weighing approximately 20 g each, were housed in groups of 5 per strain. Mice of the same strains, where indicated, were preimmunized with horse serum. Two injections of mixtures of 0.1 ml horse serum and 0.1 ml incomplete Freund's adjuvant were injected subcutaneously at 2 week intervals to each mouse. Three weeks following the first preimmunizing dose, the ascites inducing regimen was initiated.

All mice, except where otherwise indicated, received 0.5 ml intraperitoneal inoculations of equal parts of incomplete Freund's adjuvant and heat-killed 10^9 *Staphylococcus aureus* #18 cells in trypticase soy broth. Injections were administered 3-5 times at intervals of 3-5 days. Those mice receiving adjuvant alone were treated in a similar manner except that physiologic saline was substituted for the staphylococcus. In isolated instances similar booster inoculations were given 3-6 months following primary inoculation.

No prescribed schedule was followed for removal of the ascitic fluid. Mice were "tapped" at the convenience of the laboratory when their appearance indicated ascites.

Results. Fig. 1 shows results of separate experiments for 5 mouse strains and the major details of immunization and of treatment with incomplete Freund's adjuvant or *S. aureus*-incomplete Freund's adjuvant mixtures. The data shown are cumulative average yields of ascitic fluid and times of death of individual mice. The extent to which early death is responsible for poor ascites production can be judged from the data.

Striking strain differences in patterns and levels can be seen. Characteristically, CDF_1 yields are low while those for AL/N are high. With the use of *S. aureus*, good yields of ascitic fluid usually occur earlier. With adjuvant only, production is delayed for about 6 months but subsequent production can be extremely high, more than compensating for the low early yield.

The CAF_1 mouse shows reasonably high production in 2 of 3 experiments where *S. aureus* was employed. Without *S. aureus* production was delayed and remained low over the 10 month observation period.

BALB/cAnN production employing *S. aureus* was low, partially due to early fatality occurring in this strain. With adjuvant alone, however, delayed production attained high levels. These increased yields were associated with plasma cell tumors,* occurring in

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TABLE I. Strain Variation in Production of Ascites in Mice.

Strain	Total No. mice used	No. exp. done	% ascites	Ascitic fluid produced, ml: Total in period	Avg per mouse: Entire period	Mo 1–3	Mo 4–6	Mo 7–9	20% best producers of mice with ascites: Yield range, ml	% of total yield	% survival: Mo 3	Mo 6	Mo 9
Inbred strains				*9 month studies*									
BALB/cAnN	83	6	51	671	8.1	2.2	.6	5.3	31– 77	55	60	48	30
DBA/2JN	63	5	63	536	8.5	2.1	1.6	4.8	20– 92	67	87	78	64
AL/N	35	3	92	525	15.0	6.4	3.1	5.5	29– 61	54	80	54	43
C3H/HeN	7	1	86	130	18.6	2.5	10.6	5.5	38– 82	69	100	100	86
Hybrid strains													
CDF_1	35	3	46	62	1.8	.8	.8	.2	6– 17	66	66	57	51
CAF_1	63	5	55	359	5.7	4.2	1.0	.5	18– 29	45	81	76	62
AAF_1	6	1	100	103	17.1	11.5	5.6	.0	35– 35	41	33	17	17
Inbred strains				*6 month studies*									
C57BL/10ScBs	18	2	89	342	19.0	13.2	5.8	—	26–127	74	56	50	—
AKR/LwN	10	1	50	88	8.8	2.3	6.5	—	34	39	50	10	—
$C3H_fB/HeN$	16	2	50	229	14.3	2.6	11.7	—	43– 99	55	88	69	—
BRSUNT/N	16	2	25	24	1.5	1.0	.5	—	16	64	62	44	—
C57BL/10-H-2^{d}N	10	1	0	0	.0	.0	.0	—	—	—	100	60	—
STR/N	10	1	0	0	.0	.0	.0	—	—	—	100	100	—
C57L/HeN	9	1	78	109	12.1	1.4	10.7	—	22– 53	57	100	100	—
Hybrid strains													
LAF_1	16	2	38	42	2.6	.1	2.5	—	8– 30	76	75	69	—
CF_1	14	1	86	83	5.9	4.2	1.7	—	7– 55	80	86	86	—
CAF_2*	16	1	50	16	1.0	1.0	.0	—	4– 7	61	62	62	—

* CAF_2 derived from brother-sister matings of mice in a single CAF_1 litter.

10 of the 19 mice surviving beyond 6 months.

The pattern of ascitic fluid yield for the DBA/2JN mouse parallels somewhat that for the BALB/cAnN in each of the experiments. In the former strain however, mortality is less and no plasma cell tumors occurred. The pattern of earlier production of ascitic fluid employing *S. aureus* is also observed for both the strains.

Table I gives a summary of results for 17 mouse strains. The combined data shown cover several experiments for most strains. All groups except those already indicated in Fig. 1 received *S. aureus*-incomplete Freund's adjuvant mixtures.

The data shown, which cover either a 6 or 9 month period, include percentage of ascitic mice, yield per mouse in each 3 months period, and percentage of mice surviving each such period. Production by individual mice was sometimes very large: one C57BL/10 ScBs mouse produced 127 ml; a BALB/cAnN mouse, with a plasma cell tumor which produced 23 ml in the 9 month period, went on to produce 181 ml before it was sacrificed in the 11th month. Good average yields were not generally due solely to such exceptional producers; other mice in the same treatment group generally showed moderately high yields. For those mice developing ascites, the table shows the range of production by the 20% best producers and what percentage of the total yield, typically about 60%, came from these.

Discussion. Both total ascitic fluid yield and its course of production must be considered in planning use of such fluid as a source of potent antibody. The need for early production may outweigh the desirability of greater but delayed yields. Under study is the question whether increased production of fluid occurs without loss of titer or merely represents dilution.

Mice with minimal ascites could have been overlooked because they were "tapped" when their appearance indicated ascites, rather than at specified times. Also, mortality might have been less had the mice been "tapped"

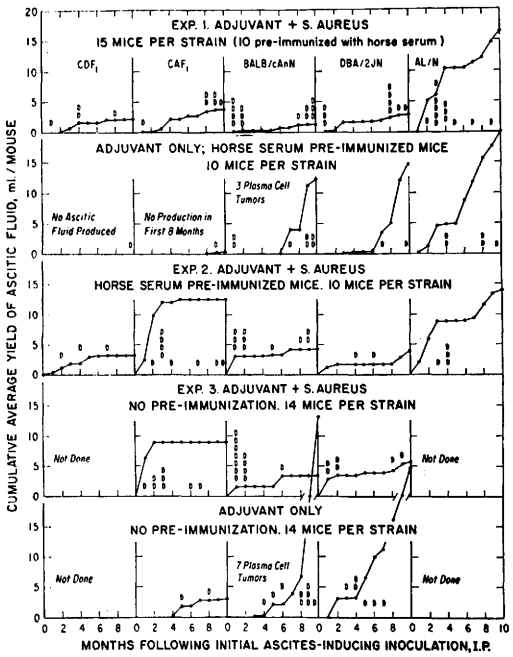

FIG. 1. Ascitic fluid production following inoculation with incomplete Freund's adjuvant alone or with heat-killed *S. aureus*. Cumulative average yields shown are based on total number of mice in experiment. Deaths of individual mice are indicated by letter *D*.

sooner and undue accumulation of fluid avoided. These alterations in technic might have led to higher incidence rates and perhaps also to higher yields than were observed.

Plasma cells have been implicated in antibody production(6) and if the property also resides in the plasma cell tumor, its occurrence in the BALB/cAnN mouse may provide a useful tool. A strain-specific carcinogenic role for the components of the adjuvant employed is suggested by the results for these mice.

Summary. Mouse strains vary in their production of ascitic fluid. Intraperitoneal injections of heat-killed *Staphylococcus aureus*-incomplete Freund's adjuvant mixtures induce early development of ascites. Yield of ascitic fluid may be greater with adjuvant alone but production is often delayed. Yield in individual mice may be very large. High late yield for BALB/cAnN mice receiving only adjuvant are associated with occurrence of plasma cell tumors.

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Red Cell Factor in Renal Damage from Hypertonic Solutions.* (26567)

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Previous work has demonstrated that the intravascular administration of certain solutions having a concentration greater than 1500 mOsm/L will produce red cell agglutination (1). This effect has been demonstrated in the lung, mesentery, brain and extremities of both cats and dogs(2). Removal of the red cell from the circulation to these areas abolishes the increase in vascular resistance which usually follows injection of markedly hypertonic media. Since the phenomenon of intravascular aggregation has been shown to be a cause of death in cardioangiography(3) and the mechanism responsible for ischemic arteriographic complications(4), we have been interested in determining its possible role in renal damage following aortography. As a first step, the following study was undertaken to delineate those changes in the kidney which result from osmotic activity alone.

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