PATHOLOGY

Increased Sensitivity to Bacterial Endotoxin of F_1 Hybrid Mice undergoing Graft-versus-Host Reaction

THE development of graft-versus-host reaction in adult F_1 hybrid mice, following the injection of parental strain spleen cells, is accompanied by a considerable increase in the phagocytic activity of the reticulo-endothelial system¹. Two weeks after injection of the spleen cells, the recipients were found to have phagocytic indices (calculated from the rate of clearance of colloidal carbon from the circulation) up to 10 times greater than normal. During investigations on the immunological reactivity of such animals to heterologous antigens², a few observations suggested that the mice were abnormally susceptible to the toxicity of relatively small doses of killed *Salmonella typhi*. Recent work has shown that stimulation of the reticulo-endothelial system in mice either by *Mycobacterium tuberculosis* (BCG) infection³⁻⁵ or by repeated injections of the yeast cell-wall preparation 'Zymosan'⁶ coincides with a profound lowering in resistance to the lethal effect of endotoxins from Gram-negative bacteria. In view of this association, F_1 hybrid mice undergoing graft-versus-host reaction were tested similarly for resistance to purified bacterial endotoxin, and an analogous effect has been demonstrated.

Male F_1 ($C57BL \times CBA$) mice, weighing 20-26 gm., were injected intravenously with 75-85 million spleen cells prepared from adult male $C57BL$ donors as described previously¹. Various dosages of *Shigella dysenteriae* type 1 endotoxin, freshly suspended in saline, were injected intravenously 14 days after the spleen cells. Previous work had shown that at this time interval stimulation of the reticulo-endothelial system was usually maximal in the strain combination used¹, although the mice appear well and indistinguishable from normal animals. The results given in Table 1 demonstrate that F_1 ($C57BL \times CBA$) mice previously injected with $C57BL$ spleen cells are 25 times more susceptible to the lethal effect of endotoxin than untreated control mice. The calculated LD_{50} 's are 11 $\mu\text{gm.}/20 \text{ gm.}$ for treated mice and 272 $\mu\text{gm.}/20 \text{ gm.}$ for control mice, with confidence limits at 5 per cent probability-level of 8-15 and 197-375 $\mu\text{gm.}/20 \text{ gm.}$ respectively. All deaths occurred within 48 hr., while those mice previously injected with spleen cells which survived dosages of 2-20 $\mu\text{gm.}$ endotoxin remained severely shocked for 24-48 hr. and then recovered. The subsequent onset and incidence of fatal graft-versus-host disease during the

following six weeks did not seem to be influenced by the endotoxin injection at day 14.

The present experiment provides a further instance, in addition to BCG infection and the effect of 'Zymosan' injection, where stimulation of the phagocytic activity of the reticulo-endothelial system has been accompanied by greatly increased sensitivity to endotoxin. The role of the reticulo-endothelial system in this state of hypersusceptibility should, however, remain *sub judice*, for there is much evidence (see refs. quoted in 5-7) supporting also a direct relationship between acquired resistance to endotoxin and an increase in the level of reticulo-endothelial activity. This phenomenon is the opposite of the one at present under consideration, although in both cases the phagocytic activity of the reticulo-endothelial system is raised.

The immunological basis for the graft-versus-host reaction has been clearly established by work on 'runt disease' induced in the young animal and of 'secondary disease' in radiation chimæras. But the immediate causes of the wasting and death which frequently attend it are at present unknown. Decreased resistance to bacterial endotoxin absorbed from the gut may be a contributory factor towards its pathogenesis.

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Immunological Differences of Deoxyribonucleoproteins from White Blood Cells of Normal and Leukæmic Human Beings

EXPERIMENTS were performed to determine: (a) whether deoxyribonucleoproteins from normal and leukæmic leucocytes react immunologically with their related control sera, or whether they cross-react with them; (b) whether they are capable of inducing formation of antibodies in rabbits; (c) whether normal deoxyribonucleoproteins cross-react with sera of rabbits immunized with leukæmic deoxyribonucleoproteins and vice versa.

Isolation of leucocytes was achieved by the method of Beck and Valentine¹. Alternatively, after sedimentation of red blood cells, citrated human plasma was centrifuged at 1,000 r.p.m. for 4 min. to recover the leucocytes. Extraction of deoxyribonucleoproteins was performed with 0.01 *M* glycine overnight after washing leucocytes with 0.01 *M* sodium citrate and 0.01 *M* glycine solution. Purification of the extract was achieved by centrifugation at 24,000g for 25 min. Isolation and extractions were made at 4° C. Details of this new method and biophysical and biochemical work on deoxyribonucleoproteins will be presented elsewhere. Antibodies were prepared by injecting intravenously a total amount of 25-30 mgm. of

Table 1. THE EFFECT OF GRAFT-VERSUS-HOST REACTION INDUCED BY INJECTION OF PARENTAL STRAIN SPLEEN CELLS ON THE SUSCEPTIBILITY OF F_1 ($C57BL \times CBA$) MICE TO BACTERIAL ENDOTOXIN

Dose of endotoxin ($\mu\text{gm.}$ per 20 gm.)	F_1 ($C57BL \times CBA$) mice	
	Controls	Animals given $C57BL$ spleen cells 14 days previously
500	8/8*	—
200	0/8	—
100	1/8	—
50	0/8	8/8
20	0/8	7/10
10	—	8/14
5	—	1/14
2	—	0/4

* No. of mice dying
No. of mice injected