CHAPTER V

DISCUSSION

Although immunity against microorganisms which interact with their host following oral ingestion is obviously complex , there is an increasing body of evidence which points to the dominant role of CMI in protecting against those organisms able to penetrate the intestinal epithelium of the host , such as Salmonella typhi (149) . Accordingly we have attempted to assess the role of host Peyer' s patches (PP) macrophages , a principal component of GALT , in the protection of mice against a virulent Salmonella strain , S. typhimurium C_5 (C_5) , which can produce mouse typhoid in susceptible strains .

Since some mouse strains are resistant to mouse typhoid (143) we utilised mice of the BALB/cJ strain , which are known to be highly susceptible to infection by C_5 .

Our approach has been to feed mice with an avirulent , auxotrophic mutant of C_5 , S. typhimurium G_{30} , previously shown by Germanier et al (1 , 2) to confer protection against mouse typhoid . Having established systems which showed adequate protection , following a massive C_5 oral challeng , we then investigated increase in both the numbers of macrophages in the PP as well as increases in the bactericidal activities to see if these could be correlated with protection .

Establishment of Mouse Protection

In our studies of mouse protection we were able to

demonstrate good protection against C_s challenge by feeding three doses of 1 x 10^{10} of G_{30} at intervals of three alternate days . Such mice were resistant to challenge by C_s 7 days after the final feeding (Table 1) , and their resistance persisted for at least 21 days .

Mice fed only one dose of 1 x 10^{10} G_{30} were susceptible to challenge by C_s at day 7 but were resistant at 21 . This delayed onset of resistance may possibly have been due to the time needed for adequate proliferation of G_{30} in GALT leading to adequate stimulation of CMI or it could be that an increase in specific antibody levels played some significant role in protection .

Although the important of cellular immunity in the acquired resistance to infections resulting from intracellular organisms such as Salmonella is well recognized the role of antibody in such immunity is far less obvious .

In the former case the work of Mitsuhashi et al (150) , Sato et al (151) , Blanden (152) have shown that macrophages from an immune host have an enhance capacity to suppress the growth of and to destroy Salmonella .

Further , Marneerushapisal and Rowley (149 , 153) and Srisart et al (7) have shown that Salmonella strains able to protect against mouse typhoid , soon appeared in the PP where they multiplied over the course of several days . Salmonella unable to proliferate in the PP did not provide protection .

Doubts as to a significant role for specific antibody in such acquired immunity arise on several counts . Notably the failure to protect a host passively by the previous transfer if immune serun or

actively by the administration of killed vaccines (154) .

However , Zinkernagel (155) observed that mice immunized with S. typhimurium were able to control a secondary typhoid infection , whereas mice immunized with Listeria monocytogenes could not do so . So that , although CMI is certainly involved in protection against these intracellular parasites , other factors such as specific antibody may also be necessary to establish a comprehensive protection . For example it has long been known that specific antibody is required for the efficent phagocytosis of Salmonella by macrophages (156 , 157) .

Macrophage Populations and Protection

Our studies have demonstrated that the feeding of G_{30} to mice was followed by an increase in the number of macrophages that could be obtained in PP suspensions . The number of such macrophages from G_{30} immunized mice was very significantly increased from that of normal mice (Table 2) . For example , when mice were fed three , 1 x 10^{10} doses of G_{30} , we obtained almost three times as many PP macrophages from day 7 onwards as could be recovered from normal mice.

Increase in the number of PP macrophages following oral feeding were clearing dose dependent (Table 2) and mirrored the in vivo protection test studies , showing a strong correlation between increase in PP macrophages and protection .

We have evidence that the PP macrophage population was also in a more activated state in the immunized animals since the PP macrophages showed enhanced esterase activity and engulfed latex

particles more avidly (Table 4) .

Role of Specific Antibody in Intracellular Killing

This study showed the absolute requirement of specific antibody for the in vitro intracellular Killing .

Not only is this antibody required for opsonization but preopsonized C_s were not killed unless additional antibody was present in the in vitro killing system . Although the mechanism by which specific antibody stimulation the killing system is not clear , that its presence is mandatory has been reported in several previous studies (9 , 158 - 160) . The triggering of the O_2 dependent mechanisms in the lysosomes (162 , 163) could explain it , and it is tempting to speculate on the possible role of activated complement complexes in disrupting these phagocytic membranes .

The optimal preopsonizing concentration of specific antibody for maximum ingestion of C_s by macrophages was about 200 ug/ml . More than 95 % of the bacteria to treated were ingested by the phagocytes. A similar concentration of immune serum produced optimal intracellular killing in our in vitro tests .

As will be seen from Table 3 the macrophages from mice fed G_{30} showed very significant increases in intracellular killing . Thus mice fed three doses of 1×10^{10} G_{30} three alternate days had then intracellular killing rate increase by 1.69 times the levels for normal mice ; this enhancement was shown at 7 days after completion of immunization and persisted at the same level for at least another 14 days . The lower single dose immunization schedule showed a 1.47 foid rate enhanced over the non - immunized control mice although

this was not detected at 7 days but only at 21 days after oral feeding .

These results correlate well with the mouse protection studies in which a single 1 x 10^{10} dose of G_{30} did not afford protection to challenge with C_s at day 7 but did at day 21 . Whereas 3 doses of 1 x 10^{10} of C_{30} afforded protection to a C_s challenge as level a day 7 and this persisted through to day 21 .

In this context it is of interest to note the observations of Van Dissel et al (143) . These workers showed that the rate of intracellular killing of virulent Salmonella typhimurium by the peritoneal macrophages of resistant CBA mice was almost 1.7 times that for macrophages harvested from the susceptible C57 BL/10 mice , this is very close to the enhancement of killing by our PP macrophages preparations following immunization requirement affording protection.

In summary this work has shown ;

a) that mice can be protected against the virulent S.typhimurium C_s strain by oral administration of the avirulent auxotroph S. typhimurium G_{30} .

b) that mice fed 3 doses of 1 x 10^{10} G_{30} at intervals become completely resistant to C_s challenge .

c) this resistance is accompanied by an approximately 3 fold increase in number of macrophages that could be harvested from the PP of protected mice compared to normal mice ; and these macrophages were activated in terms of esterase activety and uptake of latex particles .

d) that the rate intracellular killing of C_g in vitro by these activated PP macrophages is enhanced almost 1.7 times over that of macrophages from normal mice .

e) that specific antibody is mandatory in such systems for the efficient killing of C_g .

Thus our oral immunization procedure appears to stimulate CMI and circulating antibody each of which contribute to protection .