# Effect of Fumaropimaric Acid on Mouse Complement and Immunological Tolerance (35593)

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Mice have been shown to develop tolerance to soluble antigens during the neonatal period as well as after reaching immunological maturity (1). The mechanism(s) by which the immunological system can be made specifically unresponsive toward an immunogen has been a matter of speculation (2). We have previously shown that the inhibition of complement (C) by administration of a cobra venom protein (3) or by vitamin A (4) interferes with the production of tolerance. Mice which had no C hemolytic activity at the time of tolerance induction regularly were immunized while those with normal levels of C developed specific immunological tolerance (3, 4).

The present investigations were undertaken to obtain further information regarding C interaction and susceptibility to tolerance induction. A C inhibitor, fumaropimaric acid (FPA) (5) was administered concomitantly with the tolerance inducing dose of human gamma globulin. Two types of mice were used, "normal" CBA/H mice and congenic strains B10.D2 sn/J old and new lines.

*Materials and Methods.* Human gamma globulin (HGG) for tolerance production and immunization was obtained from the American National Red Cross (lot 387). A tolerance inducing dose (TID) consisted of 1 mg of aggregate-free HGG. An immunizing dose (ID) consisted of 0.1 mg of uncentrifuged HGG incorporated into incomplete Freund's adjuvant. Deaggregation of HGG was accomplished by centrifugation at 90, 000*g* for 90 min and pooling the upper two-thirds of the supernatant fluid. This preparation was then used within 4 hr after centrifugation. Immunological responsiveness was tested 7 days following the ID by administering 100 $\mu$g of $^{131}$I-HGG. Whether or not the mice developed a typical immune elimination curve was used as a criterion of whether tolerance or immunity had been produced by the injection of deaggregated HGG. Survival of the radiolabeled material was followed by performing individual body counts every second day for 1 week.

Fumaropimaric acid (5883-49T) was supplied by Doctor E. Becker. It was dissolved in a phosphate buffered saline and the final pH was adjusted at 7.8. Mice of the CBA/H and B10.D2/sn strains were used (3). Complement levels were determined at 6 and 24 hr after 10 mg of FPA were given ip. These doses were repeated at 24 and 48 hr in one group of B10.D2/sn mice. The second group of B10.D2/sn mice received FPA in the same dose for 8 consecutive days. CBA/H mice received identical doses of FPA for 4 consecutive days. Control groups of both strains were given no FPA. In another series of experiments, an attempt was made to reconstitute complement in FPA treated CBA/H mice by administration of syngeneic mouse serum complement. Since this alternative proved to be unsuccessful human serum was used instead. All mice were given a tolerance inducing dose of soluble HGG on the first day of the experiment. One group was given, in addition to the TID, 1 ml of fresh human serum every 12 hr for 4 days. A second group received a similar treatment but in addition 5 mg of FPA were given on the first and the third days. Still another group received the HGG and the FPA only on the same schedule.

In all experimental groups, the TID was

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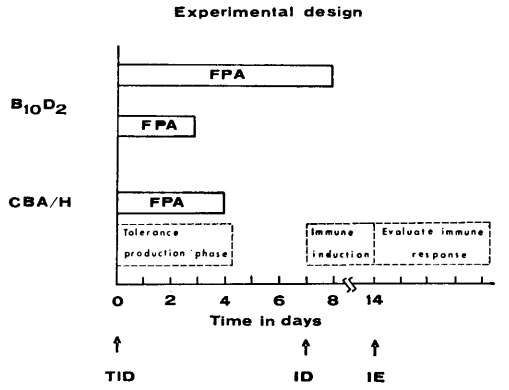


FIG. 1. Treatment administered to B10.D2 and CBA/H mice shown along the various stages of the experiment.

given iv 12 hr after the intraperitoneal administration of FPA. Control immune mice were given no TID of HGG. All mice received an ID of 0.1 mg of HGG in incomplete Freund's adjuvant on the seventh day and had an IE study beginning on day 14. Figure 1 is a schematic representation of the design of the experiment.

*Results.* Mice given an immunizing dose but no TID pretreatment of deaggregated HGG always showed immune type elimination of the $^{131}$-HGG. When a pretreatment of centrifuged HGG was given, mice regularly became tolerant to HGG. Figure 2 summarizes these observations. FPA was shown to be potent C inhibitor in the mouse and rapidly inactivated C as is indicated by a lack of circulating C dependent hemolytic

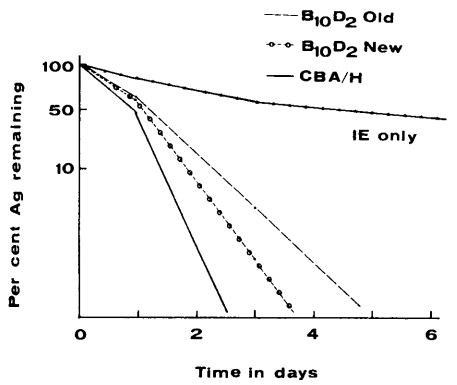


FIG. 2. Immune response on B10.D2 old, new, and CBA/H following an immunizing injection of human gamma globulin.

activity as early as 6 hr after FPA was given ip. The undetectable levels of C persisted until 48 hr following the last doses of FPA (Fig. 3). CBA/H mice given a TID concomitant to FPA administration regularly showed an immune elimination curve of radiolabeled antigen.

B10.D2/sn old line that have undetectable levels of C5 on a genetic basis developed tolerance which was quantitatively less than seen in B10.D2/sn new line that have normal C5. The curve of immune elimination on B10.D2/sn old line showed a slightly faster rate of antigen removal after a TID without FPA (Fig. 4). When the C inhibitor, FPA,

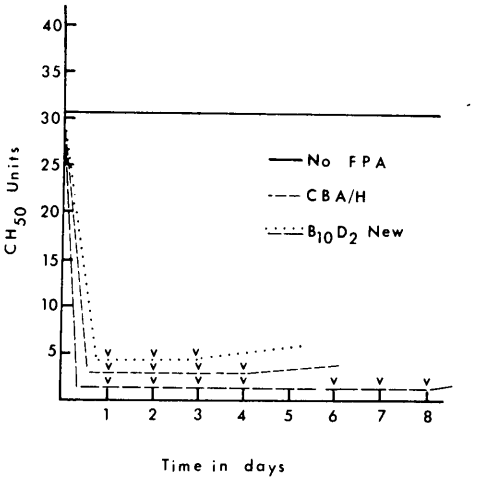


FIG. 3. Complement values following treatment with fumaropimaric acid.

was given, immunity rather than tolerance always was observed in the B10.D2/sn and CBA/H strains. All doses studied showed that B10.D2/sn old line had a somewhat higher degree of immunity than did the congenic strain whose complement system is intact (Fig. 3). Mice that were given a TID, FPA, and concomitant mouse serum complement behaved in the same way as those receiving no mouse sera. They had no complement demonstrable in serum and had an immune type of elimination; however, when fresh human serum in larger volumes was given mice became tolerant. Mice treated with FPA but without human serum developed immunity. This is shown in Table I.

*Discussion.* Production of immunological

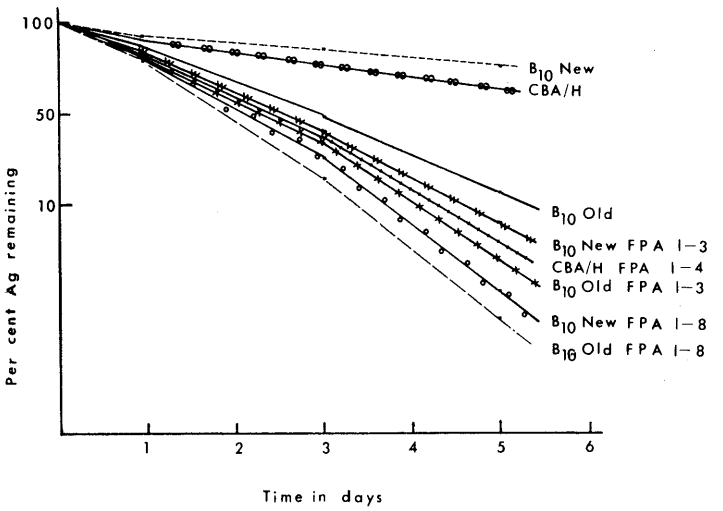


FIG. 4. Different pattern of elimination of $^{131}$I-HGG on the various mice receiving FPA on schedule shown at right.

tolerance to soluble antigens in mice seems to require the presence of an intact C system. FPA has been found to deplete guinea pig C (5) and in the studies presented here we have demonstrated a potent inhibitory action upon mouse C. Since mouse C is a rather labile system, it is not surprising to find a prolonged suppressive action on the system by the known C inhibitor.

Following the inoculation of an unaggregated preparation of HGG a tolerance inducing phase of approximately 4 days is required in order for tolerance to develop. If this phase is shortened or omitted, immunity rather than tolerance is elicited (6).

In our previous studies with cobra venom and vitamin A, we have associated depression of serum complement with loss of susceptibility to tolerance induction. Caution in interpreting these observations was exercised

TABLE I. Effect of Simultaneous Administration of Fumaropimaric Acid and Fresh Human Serum Complement in CBA/H Mice.[a]

| Hour of initial treatment | | | | | IE (% of HGG-$^{131}$I left) | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 36 | 12–24 36–48 60–72 84–96 | ID Day 7 | Day: 14 | 16 | 19 | 21 |
| CHGG 1 mg | | | 1 ml fresh human serum | 0.1 mg HGG IFA | 100 | 55 | 31 | 23 |
| | | | | | 85 | 47 | 28 | 20 |
| | | | | | 96 | 54 | 30 | 22 |
| CHGG 1 mg | FPA 5 mg | FPA 5 mg | | 0.1 mg HGG IFA | 100 | 2 | BG | BG |
| | | | | | 92 | 22 | 5 | BG |
| | | | | | 94 | 24 | 5 | BG |
| | | | | | 90 | 27 | 6 | BG |
| CHGG 1 mg | FPA 5 mg | FPA 5 mg | 1 ml fresh human serum | 0.1 mg HGG IFA | 97 | 51 | 30 | 23 |
| | | | | | 100 | 58 | 37 | 28 |
| | | | | | 95 | 51 | 29 | 21 |
| | | | | | 92 | 58 | 33 | 24 |

[a] FPA, fumaropimaric acid, given ip; CHGG, centrifuged human gamma globulin, administered iv; BG, background counts; ID, immunizing dose given in incomplete Freund's adjuvant (IFA); and IE, immune elimination.

since other possible biologic influences of the complement depressing agents had to be considered. Some of this concern was removed when one of two vehicles used for vitamin A also depressed the complement system and interfered with tolerance production while the other influenced neither system (4). The finding of another agent, fumaropimaric acid, which appears to depress complement by mechanisms quite different from those known to underlie the actions of the other complement depressing agents strengthens the case for the intimate relationship between complement activity and tolerance production indicated by the previous studies.

In the present studies, we have noted again a difference between mice having a genetically abnormal complement system and those with intact complement system in susceptibility to development of full tolerance. All of our observations to date strengthened greatly by the additional findings in the present study indicating that development of tolerance to HGG in mice requires an intact complement system. It seems likely that pursuit of this lead could contribute much to understanding of the mechanisms underlying development of immunologic tolerance. Comprehensive analysis of the underlying mechanisms and nature of immunologic tolerance are in pressing need; these results may point the direction for such analyses.

*Summary.* Inhibition of C hemolytic activity by administration of fumaropimaric acid resulted in development of immune response to tolerogenic deaggregated HGG. Mice equally treated but without FPA regularly developed immunological tolerance. From these and prior studies the conclusion can be drawn that C activity of blood is involved in some way in development of immunologic tolerance.

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