A POSSIBLE EFFECT OF ORAL TOLERANCE IN CASEIN INDUCED MURINE AMYLOIDOSIS?

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ABSTRACT

Mice raised on a normal mouse chow diet have a low immune response to immunization with casein. In addition, attempts to experimentally induce oral tolerance with casein in mice raised on a normal diet failed. Normal mouse chow contains substantial casein as a protein source. However, when mice were raised on a casein free diet and immunized with casein they had 4-5x the immune response of the normal diet mice. If such casein free mice were fed casein experimentally and then immunized the immune response was markedly suppressed. Thus mice raised on normal mouse chow are orally tolerized by the casein it contains, and this accounts for the low immune responses to casein previously reported. There are conflicting reports regarding the effect of neonatal parenterally induced tolerance to casein on susceptibility to amyloidosis, but no reports have ever considered a possible role of dietary induced tolerance in interpreting the results, a phenomenon which might well be a variable in different studies. In fact we found that one of the so called "tolerizing" protocols actually immunized the mice and prevented the development of oral tolerance due to the dietary casein. This raises the intriguing possibility that oral tolerance may be a susceptibility factor in amyloidosis.

INTRODUCTION

The phenomenon of the systemic tolerance induced by enteric exposure to antigens was first described at the turn of the century by Besredka who found that guinea pigs fed milk became unresponsive to subsequent parenteral milk challenge, and Wells and Osborne who found that guinea pigs fed corn as part of their diet could not be anaphylactically sensitized with a protein constituent of corn [1]. The phenomenon was "rediscovered" in the 1970's and both the nature of the phenomenon and its possible significance is now under intense investigation [2]. We have been studying immune response to dietary antigens for a number of years, among them bovine casein. When we attempted to induce systemic anti-casein antibodies in mice by feed-
ing we were unsuccessful. In view of the above we decided to determine if casein was an oral tolerogen, which could account for our failure to induce IgG casein antibodies by feeding.

MATERIALS AND METHODS

Mice

The mice used were BDF1 (C57B1/6 × DBA/2) females, raised in our mouse colony.

Normal Diet

The normal diet the C57B1/6 female parents were on at mating, during gestation and while nursing was Wayne Breeder B10x (Allied Mills, Inc., Chicago, Illinois). Prior to weaning the BDF1 progeny were therefore also exposed to the breeder blox. After weaning the mice were maintained on Wayne Lab. Blox.

Detection of Circulating Antibodies to Casein

One hundred μl of 125I-Casein at 0.25 μg protein/ml diluted in 1:100 normal rabbit serum, were added to 100 μl dilutions of test sera in borate buffer (0.125 M sodium borate-boric acid, 0.075 M NaCl, pH 8.3). Then 200 μl of 20% polyethylene glycol 6000 (PEG) in borate buffer was immediately added and the tubes were vortexed. After overnight incubation at 4°C, they were centrifuged for 30'. They were then decanted, drained, and washed once with 1 ml of 10% PEG. After recentrifugation and draining they were counted. Results were calculated as percentage of TCA precipitable counts bound by the test serum, corrected for the radioactivity in the precipitate of a normal control serum [3].

Oral Tolerizing Protocol

We assessed 3 different tolerizing protocols. The mice were given 20 mgm of casein by i.g. tube 1x, 4x, on consecutive days, or at 1 mg/ml in their drinking water for 28 days. To assess the induction of tolerance, one week after casein feeding was completed they received 500 μg casein in CFA i.p. and 14 days later they were boosted. After another 7 days they were bled. Antibodies to casein were assessed using a radio immunoassay and the antigen binding capacity (ABC33) in μg of casein bound per ml of undiluted serum determined.

Statistical analyses were performed using the Mann-Whitney "U" test.

RESULTS

As can be seen from Table 1, our initial attempts to produce orally induced tolerance with casein in BDF1 mice failed, suggesting that casein was not an oral tolerogen. However, the antibody levels were relatively low, and we had come across the statement in one of Clerici's papers on the possible role of the immune system in casein induced amyloidosis, that "the mouse...is such a poor producer of anti-casein antibodies that they are hardly, if at all, detectable in the serum..." [4]. Thus it occurred to us that if there was casein in normal mouse chow, all mice raised on normal chow might be "pretolerized" by the dietary exposure. Indeed, we discovered that both Wayne Breeder Blox and Wayne Lab Blox contained substantial casein as a protein source. Therefore we repeated the experiments, but this time with mice that had been bred and raised in such a way that