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Oral Tolerance in Birds and Mammals: Digestive Tract Development Determines the Strategy¹

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Primary Audience: Researchers, Immunologists, Veterinarians

SUMMARY

Development of oral (or airway) vaccines to be used for the vaccination of poultry is clearly a necessity because the process of individual bird vaccination is time-consuming and costly. One of the key issues to resolve in this context is whether the vaccine is protected from oral tolerance. Oral tolerance is a form of immune tolerance that is induced following the feeding of protein antigens dissolved in water or aqueous buffers. The issue is particularly relevant in cases in which nonpathogenic subunit protein vaccines are considered as vaccines. These short peptides/proteins contain the immunogenic moiety of the parent pathogen, but they lack the capacity to induce inflammation, and an antigen in the absence of inflammation might induce tolerance. The following review summarizes our main observations on mechanisms of oral tolerance in the chicken, compares these findings to what is known in the mammal, and provides some insight for developing strategies for oral vaccination of poultry.

Key words: oral tolerance, immune response, dietary protein, chicken, mouse

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INTRODUCTION: ORAL TOLERANCE

Receptors for antigen on lymphocytes are randomly generated during ontogeny. A central model describing the development of the adaptive immune system describes these receptors as diverse (namely, they recognize any antigen pattern), specific (namely, each receptor will bind a single antigenic epitope), and clonally distributed [1]. Although native clonotypes are randomly generated, receptors capable of recognizing many innocuous antigens are generated as well, including self and harmless nonself epitopes [2]. Because responses to self and harmless nonself are expected to lead to autoimmunity and allergy, respectively, several means exist to actively prevent these undesired consequences [3]. Silencing rogue clones is defined as tolerance, and tolerance may be imposed in central and peripheral lymphoid organs [3]. A unique form of tolerance, oral tolerance, is observed following the exposure of mammals to oral innocuous antigens [4, 5]. Thus, feeding an animal

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with protein antigens, usually dissolved in an aqueous vehicle, often results in immune nonresponsiveness that has been characterized in terms of dosage [6], mechanism [7], cell types involved [8, 9], and cytokine expression [10]. Oral tolerance to oral (dietary proteins) is dominant in mammals; namely, it is difficult to induce adaptive immune responses to these antigens once tolerance is established [11]. Interestingly, oral tolerance is limited in duration and will persist as long as antigen is provided via the oral route [11]. Not surprisingly, oral tolerance has been the focus of 2 independent lines of research. First, for development of oral vaccines it has become important to insure that a given vaccine candidate will not inadvertently lead to tolerance [12, 13]. Second, oral tolerance is a potential therapeutic treatment of autoimmune diseases. In the latter case putative auto-antigens have been given to patients in clinical trials with the hope that such an approach will reintroduce tolerance to self. The results of these trials, however, remain inconclusive [14].

**ORAL TOLERANCE IN THE CHICKEN?**

Development of oral (or airway) vaccines to be used for the vaccination of poultry is clearly a necessity because the process of individual bird vaccination is time-consuming and costly. Furthermore, if vaccination could be practiced in-ovo during incubation in hatcheries, other advantages could be realized. For example, in-ovo vaccination will guarantee the vaccination of each embryo (oral vaccination of a population cannot provide this with certainty), and vaccination programs could be centralized with further cost reduction. To develop these strategies several biological issues need to be addressed: is the hatchling or embryo immunologically mature? To what extent will maternal antibodies prevent or block vaccination? Will the vaccine be protected from oral tolerance? In the last case oral vaccines constructed from live or killed pathogens have been shown to immunize without exception. The question arises, however, in cases in which nonpathogenic subunit protein vaccines are considered as vaccines. These short peptides/proteins contain the immunogenic moiety of the parent pathogen, but they lack the capacity to induce inflammation; an antigen in the absence of inflammation might induce tolerance [2].

With these issues in mind we set out to investigate conditions for generation of oral tolerance in chickens [15]. Most surprisingly, we observed that protein antigens dissolved in water were potent oral immunogens in the chicken and turkey. We clearly demonstrated that oral protein antigens (albumins, β-lactoglobulin, and bovine hemoglobin) induced robust antibody responses in the absence of any added adjuvant and that these antibody responses were detected in both serum and bile. Characterization of this response revealed that 1) a daily feeding regimen was more immunogenic than single dose feedings; 2) by using a daily feeding regimen, as little as 2 mg/chick per day was fully immunogenic; 3) effective immunization was attained in chicks older than 10 d of age (100% responders); 4) although potent, the response declined quite rapidly after cessation of feeding; 5) the main antibody class in the serum was IgG, whereas IgA was undetectable; and 6) high IgA levels were detected in the bile but only after an administration of a booster feeding regimen.

Interestingly, we observed that only proteins dissolved in water were immunogenic, whereas if the same protein was offered as dry matter it was immunologically ignored. What makes the water-soluble form immunogenic, whereas the dry form is ignored? Assuming the response is generated in the hindgut, we proposed that the basis for this distinction is explained by the way the avian gut processes these 2 forms of antigen. Solid antigen is retained for longer periods and undergoes extensive degradation, whereas liquid antigen is retained for shorter periods and undergoes less extensive degradation: residence time of solids in the crop and gizzard of the 6-wk-old chick was 4 to 5 h for solids, whereas liquids had a mean residence time of less than 3 h [16]. The time of passage through the small intestine was approximately 2 h and is 15% faster for liquids than for solids. The time of exposure to proteolytic enzymes determines the amount of proteolysis, and in particular, the shorter duration in the gizzard will decrease the amount of peptic digestion when soluble proteins are fed. In chicks fed casein, as compared with soybean meal—a less soluble protein—more high molecular weight peptides were observed in the small
intestine with the more soluble protein [17]. Thus, when soluble proteins are fed it would be expected that more high molecular weight protein fragments will reach the large intestine and that these would be stimulatory under the inflammatory conditions prevailing in the hindgut [18]. Solid antigen would be processed and absorbed in its majority, and the antigen, if any, which reaches the hindgut, would be fragmented and nonimmunogenic.

The fact that a daily intake of a protein solution was immunogenic in the chicken and turkey was problematic from a conceptual immunological point of view. As introduced above, a basic premise of contemporary immunology is that noninfectious self or nonself should not evoke adaptive immune responses [19]. A common understanding of gut-associated lymphoid system (GALT) function states that oral tolerance might serve as a mechanism to protect the host from immune responses against innocuous food-derived antigens [4]. In contrast, we observed that such proteins were not ignored, nor did they induce tolerance, but rather they were potent immunogens. The practical aspect of these observations was that in the correct context, any oral protein might lead in the chicken to the formation of food allergy. Finally, the fact that oral proteins were immunogenic in the chick and tolerogenic in mammals inevitably led to the necessary comparison between bird and mammal in terms of mucosal immunity.

CHICKEN AND MAMMAL—DIFFERENT LIFESTYLES LEAD TO DIFFERENT MUCOSAL STRATEGIES

The chicken is defined as a precocial-type bird (as opposed to altricial birds that hatch with eyes closed, little or no down, and are wet-nursed by their parents until they gain independence). Precocial birds hatch with eyes open, covered with down, and are immediately independent. In nature, their degree of development is defined as level 2; namely, they forage independently but do so while following their parents (imprinting). The industrialized chick is at level 1 of precocial development, namely it develops totally independent of parents. After clearing the shell, chicks immediately begin foraging; this immediate exposure to adult-type food leads to the rapid colonization of the gut by adult-type microflora. In terms of numbers, the gut flora levels are at a maximum within 24 to 48 h posthatch, although fluctuations in bacterial populations might occur [20]. Consequently, the gut is subjected to vast morphological and functional changes during the posthatch period, which result in increased intestinal absorptive surface and rapid relative mass [21, 22].

The development of the mammalian digestive system is different in terms of nutrition and rate. The mammal is exposed to adult-type food only after weaning. At this time the gut undergoes morphological changes as well as microfloral changes to adapt to the changing of diet. Another important difference refers to the state of GALT development at the time the gut is colonized by adult bacteria and is exposed to adult food. In the chick, this development occurs during the first 3 d of life, a period in which the intestinal immune system is far from being functional [23, 24]. In the mouse, for example, the parallel development occurs at 4 wk of age, a time at which most of the adaptive immune system is functional.

Due to the fundamental differences in lifestyle and digestive tract development, we suggested that response or tolerance toward oral antigens in the chicken might be age dependent, namely that tolerance develops during the period when GALT has yet to mature and the gut allows the absorbance of macromolecules. This hypothesis was supported by studies indicating that an immature immune system was more sensitive to generation of oral tolerance [4]. When testing this hypothesis [25], we observed that newly hatched chicks did develop oral tolerance when fed antigen in dissolved and dry forms. The window for tolerance induction was very narrow and lasted only 3 d posthatch [26]. Whereas secondary responses to parenteral immunizations were only moderately depressed, the responses to enteral immunizations were absolutely depressed. This indicated that oral tolerance was in fact a state of immune balance that could be overridden in cases of severe inflammatory stimuli. Hence, tolerance is a quantitative trait balanced by opposing signals, and the final outcome reflects a regulatory balance between stimulation and suppression [5]. To further es-
tablish the immunological properties of chicken oral tolerance, we showed that it was antigen-specific, dose-dependent, and that it was not permanent if antigen was withdrawn [25, 26].

The chicken is not different, therefore, from the mammal in the sense that oral antigen induces tolerance, nor in the sense that continuous exposure to oral antigen might lead to immune response, rather than tolerance in several species, such as the rabbit or hamster [27, 28]. What is different is the period in which these species are sensitive to tolerance induction. In the chicken tolerance is generated within the first week of life, whereas in the mammal tolerance can be induced for much longer periods, and more successfully so in weaned animals. A possible explanation for this difference is deduced from the different lifestyles of the 2 species. The bird feeds from the environment immediately from hatch and, thus, is immediately exposed to food antigens during the period in which the immune response is immature and can be rendered tolerant (tolerized). Because the diet is usually the same at later ages, with no new antigens relative to those at the time of hatch, there would be no need for continuing tolerance, and thus the adult bird is programmed to respond. In the mammal, however, the change of diet after weaning exposes it to a completely new set of innocuous food antigens at a time when the immune system is almost fully mature. Hence, there would be a need for extended tolerance, and thus the adult bird is programmed to respond. The mechanism for oral tolerance in mammals is probably a result of tolerant regulatory (T_{reg}) cell function in the intestine; this means that this is the result of what is defined as peripheral tolerance, and tolerance is a balance between helper and suppressor T cell circuits [29].

The mechanism for oral tolerance in the chicken has yet to be determined. What can be excluded at this time, however, is a classical mechanism for clonal deletion [5] because tolerance to BSA was lost by 100 d of age in chicks fed a single bolus on the day of hatching [26]. This indicated that maintenance of tolerance to dietary antigens requires continued exposure to the same antigen throughout life. Though not yet extensively tested, the fact that intermittently fed chicks were still tolerant to BSA by 100 d of age supports this notion. Hence, other temporary mechanisms of tolerance such as anergy (loss of response due to clonal paralysis), suppression, or immune deviation could be considered at this time [6–8]. Oral tolerance in chickens could depend on active or passive processes in the gut. Active processes imply age-related development of various cell types in GALT (suppressor or regulatory T lymphocytes and antigen-pres-enting cells) that downregulate immune responses to oral antigens [27, 28]. Therefore, oral tolerance would result from local immune-regulatory circuits.

Passive processes, on the other hand, reflect the development of the mucosal barrier and its accessibility to oral antigen [31, 32]. In this case, oral tolerance would be the result of a central mechanism evoked by the passage of intact antigen, via the circulation, to central lymphoid organs (thymus, bursa, and spleen) and particularly so during the period in which the development of the lymphoid repertoire occurs [33]. Our findings indicate a central tolerance mechanism as responsible for the tolerance to oral antigen in the newly hatched chick for the following reasons: (a) the period in which tolerance was invariably induced correlates with the period re-
quired for the completion of the intestinal barrier [22, 34, 35] and occurs prior to the maturation of GALT in the chick [23, 24]; (b) tolerance was demonstrated locally in the gut and systemically [25]; (c) intravenous injection of BSA-induced tolerance in young chicks in a manner similar to that of oral antigen [26]. The significance of this observation is emphasized by the fact that the dosage required to generate IV tolerance was calculated to be similar to that absorbed following feeding, namely up to 2% of ingested antigen [36]. (d) Finally, tolerance was totally abrogated in chicks carrying maternal antibodies specific for BSA. As these experiments do not indicate the systemic site(s) of tolerance induction, all central lymphoid organs (thymus, spleen, and bursa) could be involved in the process.

During the period the immunologically immature chick is exposed to innocuous food antigens, it is also exposed to potentially hazardous antigens and pathogens that should not lead to generation of tolerance, and indeed do not. Several possible mechanisms could explain the divergence of outcome following exposure to the innocuous or harmful: tolerance or response could reflect the stimulatory properties of the antigens in the gut (i.e., dosage or frequency of exposure [6, 8]); alternatively, tolerance could be specifically avoided by potential inhibitors of tolerance, such as maternal antibodies. The capacity of maternal antibodies to block circulating antigens (natural or vaccine-derived), thereby preventing disease or blocking early interaction with immune cells [26], indicated that they might also be relevant in prevention of tolerance [37]. This possibility was confirmed by showing that presence of anti-BSA maternal antibodies blocked generation of oral tolerance to BSA. Classically, maternal antibodies protect the hatchling against environmental pathogens during the period required for the immune system to mature. In addition, we suggest that at the same time they prevent generation of tolerance to the same antigens by blocking their access to central lymphoid organs. Because the dam does not produce antibodies to food-derived innocuous antigens, the relevant maternal antibodies are absent, and tolerance to food antigens is efficiently generated in the chick. Thus, the harmful entity is prevented from causing damage and inducing tolerance whereas the innocuous entity is ignored or is a generator of tolerance.

**CONCLUSIONS AND APPLICATIONS**

1. Oral tolerance to protein antigens may be induced in 1- to 3-d-old chicks.
2. After this age, protein antigens induce robust responses rather than tolerance.
3. During 1 to 3 d of age, protein antigens are absorbed and are recognized (blocked) by circulating maternal antibodies.
4. Therefore, it would not be advisable to use subunit protein vaccines as oral vaccines in the chick prior to 3 d of age because they might be blocked by maternal antibodies or they might induce immune tolerance.
5. Oral vaccines should be designed to overcome these handicaps.

**REFERENCES AND NOTES**


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