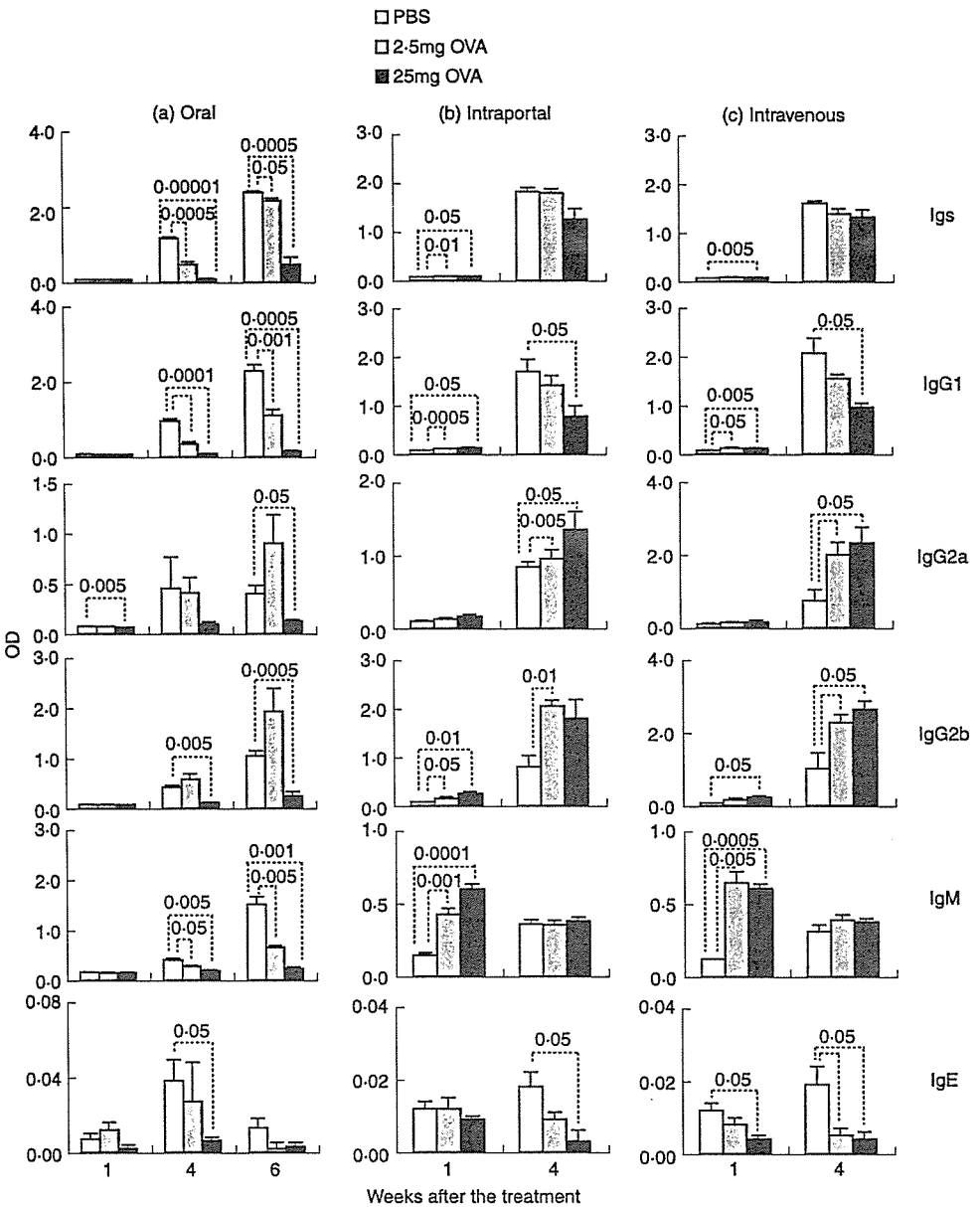

Figure 8. Production of anti-OVA immunoglobulins is suppressed by the oral administration of intact OVA, but not by intraportal or intravenous injection of intact OVA. BDF_1 mice were orally administered (a) or injected into the portal (b) or peripheral vein (c) with 2·5 mg or 25 mg of OVA or PBS. The mice were i.p. immunized with OVA plus alum 1 and 3 weeks after the first oral administration or injection of intact OVA. Orally treated mice were additionally i.p. immunized with OVA 5 weeks after the first oral administration. Peripheral blood was collected at various weeks. Anti-OVA immunoglobulins in plasma were assessed by ELISA. Data are expressed as the mean of OD (415 nm) + SE of five to six mice. Each value represents statistical significance ($P <$), compared with control mice treated with PBS in the same way.

vessels in the small intestine. Some allergens have been shown to cross the mucosal barrier by the disruption of tight junctions.[38,39] Notably, it has been shown that soluble protein antigens are rapidly pinocytosed by enterocytes and co-localized with major histocompatibility complex (MHC) class II in a vesicular compartment.[40] Bland *et al.* proposed that intestinal epithelial cells (IECs) presented antigens on their surface to local T cells[41] and induced suppressor T cells.[42] Recently, it has been reported that tolerosomes, which have an exosome-like structure and carry MHC class II with antigens, were released from IECs into serum.[43] It has also been shown that tolerosome from mouse serum 1 hr after the oral administration of OVA-induced oral tolerance and this induction was MHC class II dependent.[44] Hereafter, it is necessary to examine whether the 45 000 MW OVA antigens detected are free antigens or are included in exosomes in mouse serum.

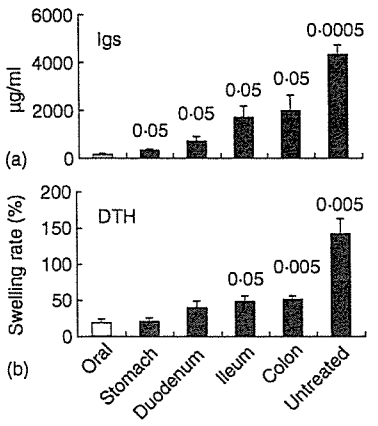

Figure 9. Less effective induction of immunological tolerance by injection of intact OVA into lower intestinal tract. BDF_1 mice were orally administrated or injected into the guts with 25 mg of OVA. OVA-treated and untreated mice were i.p. immunized with OVA plus alum 1 and 3 weeks after OVA treatment. Peripheral blood was collected 1 week after the second immunization of OVA plus alum. Plasma anti-OVA immunoglobulin was assessed by ELISA (a). DTH response against OVA was assessed 6 weeks after the second immunization (b). Data are expressed as the mean + SE of 3–9 mice. Each value represents statistical significance ($P <$), compared with mice orally administered with OVA.

It has been demonstrated that CD25-positive cells are crucial to immunological suppression by the transfer of serum after the oral administration of OVA.[32] The liver has been shown to contribute to tolerance induction because the intraportal injection of allogeneic cells[24,25] eggs of a parasite[26] or insoluble protein[27] induces immunological tolerance against the antigen. It is reported that liver endothelial cells endocytose OVA by a mannose receptor, CD206,[45,46] and antigen presentation by cells induces T-cell tolerance against OVA.[46] In this study, however, the injection of intact OVA into the portal or peripheral vein did not induce immunological tolerance but rather enhanced part of OVA-specific antibody production. As mannose receptors are also expressed on macrophages in red pulp in the spleen[47] intact OVA may be captured by macrophages in the spleen after intravenous injection. In our experimental system, these antigen-presenting cells (APCs) in the liver and spleen may not induce immunological tolerance when they endocytose intact OVA.

The uptake of intact antigens untreated with digestive enzymes may lead to immunological enhancement such as allergy. Ileal injection of BSA treated with pepsin induces immunological tolerance against BSA, whereas ileal injection of intact BSA enhances anti-BSA responses.[48] Correspondingly, in this study, the injection of intact OVA into the ileum or colon significantly enhanced both OVA-specific antibody production and DTH response. Induction of oral tolerance was more difficult when intact OVA was injected into the lower intestinal tract. It is reported that the impairment of gastric digestion of caviar extracts significantly enhanced caviar-specific IgG1, IgG2a, and IgE levels in mice.[49] In addition, cod proteins treated with pepsin show reduced IgE-binding capability and reduced histamine release from human basophils.[50] In the previous study, we demonstrated that oral tolerance against sheep red blood cells (SRBCs) was induced in young mice but rather SRBC-specific antibody response was enhanced in aged mice by the oral administration of SRBC.[51] Digestive and absorptive capacity is decreased in elderly people.[52] Reduced digestive capacity in aged mice might result in the failure of oral tolerance induction. In this report, it was shown that the absolute gastrointestinal ingestion of OVA via the upper gastrointestinal tract is crucial for oral tolerance induction. As macromolecular OVA antigens but not digested antigen fragments are detected in tolerant-mice serum, not only digestion but also some modification of macromolecular OVA in the gastrointestinal tract may be essential for oral tolerance induction.

The detection of OVA antigens has been shown in mouse serum 1 hr after the oral administration of OVA, and serum transfer induced significant suppression of OVA-specific immune responses.[31] Recently, it was shown that tolerosomes including MHC class II are produced by IEC at 1 hr after the oral administration of OVA, and tolerosomes induce oral tolerance.[44] Also in our results, OVA antigens were remarkably detected at 30 min and 1 hr after the oral administration of OVA.

In this study, it was clearly demonstrated that the absolute gastrointestinal ingestion of OVA via the upper gastrointestinal tract is crucial to the establishment of oral tolerance. Although macromolecular OVA antigens are detected after the oral administration of OVA in tolerant-mouse serum, the injection of intact OVA cannot induce tolerance. Therefore, some modification of macromolecular OVA in the gastrointestinal tract and ingestion may be essential for oral tolerance induction.

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