

GASTROINTESTINAL INFLAMMATION

Gastrointestinal *Candida* colonisation promotes sensitisation against food antigens by affecting the mucosal barrier in mice

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Backgrounds and aims: Controversy still exists as to whether gastrointestinal colonisation by *Candida albicans* contributes to aggravation of atopic dermatitis. We hypothesised that *Candida* colonisation promotes food allergy, which is known to contribute to a pathogenic response in atopic dermatitis. We tested this using a recently established murine *Candida* colonisation model.

Methods: *Candida* colonisation in the gastrointestinal tract was established by intragastric inoculation with *C. albicans* in mice fed a synthetic diet. To investigate sensitisation against food antigen, mice were intragastrically administered with ovalbumin every other day for nine weeks, and antiovalbumin antibody titres were measured weekly. To examine gastrointestinal permeation of food antigen, plasma concentrations of ovalbumin were measured following intragastric administration of ovalbumin.

Results: Ovalbumin specific IgG and IgE titres were higher in BALB/c mice with *Candida* colonisation than in normal mice. Gastrointestinal permeation of ovalbumin was enhanced by colonisation in BALB/c mice. Histological examination showed that colonisation promoted infiltration and degranulation of mast cells. *Candida* colonisation did not enhance ovalbumin permeation in mast cell deficient W/W^v mice but did in congenic littermate control +/+ mice. Reconstitution of mast cells in W/W^v mice by transplantation of bone marrow derived mast cells restored the ability to increase ovalbumin permeation in response to *Candida* colonisation.

Conclusions: These results suggest that gastrointestinal *Candida* colonisation promotes sensitisation against food antigens, at least partly due to mast cell mediated hyperpermeability in the gastrointestinal mucosa of mice.

Candida albicans is part of the indigenous microbial flora of the human gastrointestinal tract. In healthy individuals, populations of this fungus pose no threat to the host. However, in hosts receiving antibiotic treatment,¹ immunocompromised states,^{2–4} and occasionally in apparently healthy persons,⁵ elevated populations can pose a significant risk.⁵ In addition, it has been hypothesised that excessive colonisation by *C. albicans* in the gastrointestinal tract may constitute aggravating factors in atopic dermatitis (AD), but this remains controversial.^{4–6–7} AD is a chronic, relapsing, highly pruritic inflammatory skin disease with multifactorial causes, such as susceptibility genes, conditions within the host environment, and immunological factors.⁸ To date, laboratory and clinical investigations have demonstrated that IgE mediated food allergy plays a pathogenic role in a subset of patients with AD.^{9–11} Some reports have shown increased gastrointestinal permeability in AD patients.^{12–14} Hyperpermeability of the gastrointestinal mucosal barrier results in enhanced transport of intact and degraded antigens across the gastrointestinal mucosal barrier, which could favour food protein sensitisation and food allergy in susceptible individuals.¹⁵ We therefore hypothesised that gastrointestinal colonisation by *C. albicans* may be involved in aggravation of AD by affecting the mucosal barrier in a manner that results in increased permeation of food allergens and subsequent manifestation of a food allergy.

Most models of gastrointestinal colonisation by *C. albicans* have used oral inoculation of *C. albicans* in adult mice treated with antibiotics and immunosuppressive agents,^{16–21} or in infant mice.^{22–23} These treatment regimens have been necessary because competitive indigenous bacterial flora and the immune system prevent colonisation by *C. albicans*.^{17–19–24}

However, given that *C. albicans* is indigenous to the gastrointestinal tract of healthy humans, such methods of administration, particularly the immunosuppressive route, should be avoided so that an animal model that is typical of gastrointestinal colonisation by *C. albicans* can be developed and the relationship between gastrointestinal *Candida* colonisation and allergic responses can be studied further. We recently reported a model of sustained gastrointestinal *Candida* colonisation by a single intragastric inoculation of *C. albicans* in healthy adult mice without administration of antibiotics or immunosuppressants.²⁵ This was achieved by feeding mice a synthetic diet that resulted in reduced numbers of lactobacilli in the stomach. While animals show a good healthy appearance in this model, high faecal recovery of *C. albicans* is still observed at least 18 months after the single inoculation (unpublished data), suggesting asymptomatic *Candida* colonisation in the gastrointestinal tract. Therefore, using this model it was possible to investigate the role of gastrointestinal colonisation by *C. albicans* in the sensitisation against food allergens.

To test our hypothesis, we initially determined whether sensitisation against food antigens was promoted in mice with gastrointestinal tracts that had been chronically colonised by *C. albicans*. We then assessed the effect of gastrointestinal *Candida* colonisation on permeation of food antigens. Finally, because the barrier and transport properties of the gastrointestinal epithelium are actively regulated by

Abbreviations: AD, atopic dermatitis; BMMC, bone marrow derived mast cells; BSA, bovine serum albumin; HRP, horseradish peroxidase; OVA, ovalbumin; PAS, periodic acid-Schiff; PBS, phosphate buffered saline; TLR, toll-like receptor; TNF, tumour necrosis factor

mast cells which are activated in response to various pathogen associated stimuli,^{26, 27} we investigated whether mast cells were involved in the permeation of food antigens in mice with gastrointestinal *Candida* colonisation.

MATERIALS AND METHODS

Animals

Specific pathogen free female five week old mice were used in all experiments. BALB/c mice were purchased from Charles River Japan (Yokohama, Japan). Mast cell deficient mice (WBB6F1-W/W^v) and congenic littermate control mice (WBB6F1-+/+) were purchased from Japan SLC (Hamamatsu, Japan). All mice were housed in a temperature controlled ($23 \pm 2^\circ\text{C}$) room with a dark period from 20:00 to 08:00 and allowed free access to water and a purified diet prepared according to AIN-93G.²⁸ The study was approved by the Hokkaido University Animal Use Committee, and animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University.

Inoculation and enumeration of *C albicans*

C albicans (JCM 1542) was obtained from Japan Collection of Microorganisms of the Institute of Physical and Chemical Research (Saitama, Japan) and maintained as previously described.²⁵ For inoculation, all mice were acclimatised to the purified diet for two weeks before being deprived of the diet for 16 hours. Mice were then inoculated intragastrically with 0.2 ml of saline containing 1×10^8 cells of *C albicans*. Control mice were intragastrically administered 0.2 ml of the vehicle. Faecal specimens and tissue homogenates were quantitatively cultured using a standard pour plate technique, as previously described.²⁵

Oral immunisation experiment

At three weeks after inoculation, oral immunisation with ovalbumin (OVA) was started in BALB/c mice. Phosphate buffered saline (PBS 0.2 ml) containing 0.1 mg of OVA (grade V; Sigma, Missouri, USA) was intragastrically administered every other day for nine weeks. Blood samples were obtained from the tail vein at weekly intervals and subjected to ELISA for measurement of OVA specific antibody titres, as described below. In addition, every week, faecal specimens were analysed for number of *C albicans* organisms, as described above. On the last day of the experiment, mice were anaesthetised with diethyl ether and sacrificed by exsanguination from the carotid artery. Following laparotomy, the stomach, jejunum, ileum, and colon were excised, homogenised in PBS, and the number of *C albicans* organisms were counted, as described above.

Gastrointestinal permeation experiment

Gastrointestinal permeability was determined in vivo by measuring the appearance in blood of horseradish peroxidase (HRP) and OVA, administered by gavage according to Wang and colleagues²⁹ and Saitoh and colleagues,³⁰ respectively, with some modifications.

At four to five weeks after inoculation, mice were deprived of the diet for 12 hours, after which 0.6 ml of PBS containing 0.6 mg of HRP (Sigma) or 0.2 ml of PBS containing 2 mg of OVA were intragastrically administered. Blood samples were then collected from the tail vein at 0, 30, 60, and 120 minutes after administration, and plasma HRP and OVA concentrations were measured by ELISA, as described below. Thereafter, mice were anaesthetised with diethyl ether and sacrificed by exsanguination from the carotid artery. Following laparotomy, the stomach was excised, opened along the greater curvature, washed with ice cold saline, and

embedded in OCT compound (Sakura Finetechnical, Tokyo, Japan) for histological examination.

ELISA

For determination of OVA specific IgG titre in the sera of mice, 96 well microtitre plates (Corning, New York, USA) were coated overnight at 4°C with 200 $\mu\text{g/ml}$ OVA in 50 mmol/l carbonate buffer, pH 9.6. Plates were blocked with PBS containing 1% bovine serum albumin (BSA, fraction V; Serologicals Proteins, Illinois, USA) (PBS-B) at 37°C for one hour. Test sera serially diluted with PBS containing 0.2% BSA and 0.02% Tween 20 (PBS-BT) were then added and incubated at 37°C for two hours. After incubation, HRP conjugated goat antimouse IgG (Zymed, California, USA), diluted 1:2000 in PBS-BT, was added and incubated at 37°C for one hour. Wells were washed five times with PBS containing 0.02% Tween 20 (PBS-T) between each step. Plates were developed at room temperature after addition of *o*-phenyldiamine (0.4 mg/ml) and hydrogen peroxide (0.012%) in 7 mmol/l citrate buffer, pH 5.0. Finally, 1 mol/l H_2SO_4 was added, and absorbance was measured at 490 nm with a microplate reader (model 550; Bio-Rad, California, USA). Preimmunised serum was used as a negative control. The average extinction in negative control wells, to which three times the standard deviation was added, provided the reference for determination of the titre in the test sera. Antibody titres were expressed as the reciprocal of the last dilution yielding an extinction value higher than the reference value.

For determination of the OVA specific IgE titre, 96 well microtitre plates were coated overnight at 4°C with 10 $\mu\text{g/ml}$ antimouse IgE monoclonal antibody (LO-ME-2; Zymed) in 50 mmol/l carbonate buffer, pH 9.6. Plates were blocked with PBS-B at 37°C for one hour. Test sera serially diluted with PBS-BT were then added and incubated overnight at 4°C . After incubation, 20 ng/ml OVA-DIG conjugate in PBS-BT was added and incubated at 37°C for one hour. Coupling of DIG to OVA was performed using a DIG protein labelling kit (Roche Diagnostics, Tokyo, Japan) according to the manufacturer's instructions. HRP conjugated sheep anti-DIG Fab fragments (Roche Diagnostics), diluted 1:4000 in PBS-BT, was then added and incubated at 37°C for one hour. Wells were washed five times with PBS-T between each step. Plates were developed at room temperature after addition of 3,3',5,5'-tetramethylbenzidine (0.123 mg/ml; Sigma) and hydrogen peroxide (0.012%) in 2 mol/l citrate buffer, pH 5.0. Finally, 1 mol/l H_2SO_4 was added, and absorbance was measured at 450 nm with a microplate reader. Antibody titres were determined as described above.

To measure plasma HRP concentrations, 96 well microtitre plates were coated with goat anti-HRP antibody (Sigma) for two hours at 37°C . All subsequent steps were performed at 37°C with extensive washing between steps. Plates were blocked with 1% BSA for two hours. Diluted plasma samples in PBS-BT were added to the wells and incubated for two hours. Plate development and measurement were as described for the OVA specific IgE.

Plasma OVA concentration was measured by sandwich ELISA using anti-OVA IgG (Chemicon, California, USA) and HRP conjugated rabbit anti-OVA IgG (Rockland, Pennsylvania, USA) for capture and detection antibodies, respectively, as described previously.³⁰

Histology

Cryostat sections (5 μm) of stomach were prepared and stained with haematoxylin and periodic acid-Schiff (PAS) reaction for detection of *C albicans* or with toluidine blue for identification of mast cells. The number of mast cells in the stomach was counted using a high power field in a section

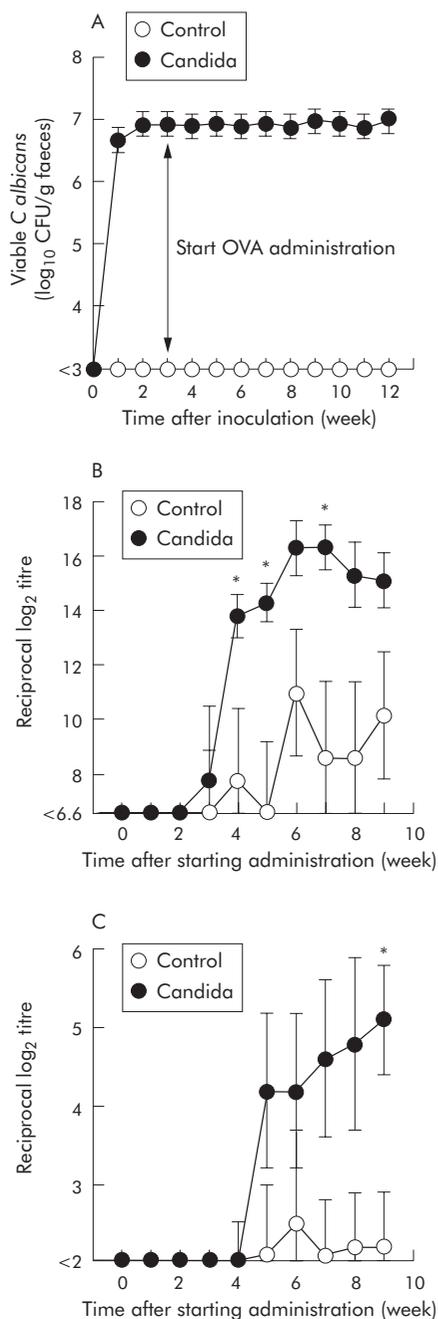


Figure 1 Time course of changes in recovery of organisms from faeces and titres of serum antibodies specific to intragastrically administered ovalbumin (OVA) in BALB/c mice with and without gastrointestinal *Candida* inoculation. The number of viable *C. albicans* in faeces (A) was determined weekly by quantitative culture method. Anti-OVA IgG (B) and IgE (C) titres were measured in sera taken at the times indicated after starting intragastric administration of OVA. Values are means (SEM) of six mice per group. * $p < 0.05$ compared with control mice without *Candida* inoculation at each time point.

from each specimen. According to Nakajima and colleagues,³¹ with some modifications, the number of mast cells in the gastric mucosa was counted in a high power field measuring 0.65 mm² using a light microscope equipped with a 10 \times objective and a 10 \times eyepiece containing a reticule (Olympus, Tokyo, Japan). The counting areas included stratified squamous epithelium, lamina muscularis mucosa, and submucosal layer in the forestomach, and gastric pits and fundic glands in the glandular stomach. The cell with stained

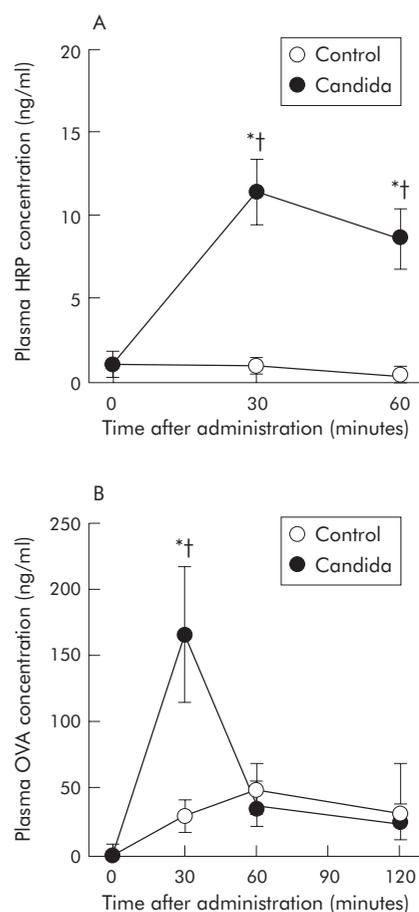


Figure 2 Time course of changes in plasma concentration of intragastrically administered proteins in BALB/c mice with and without gastrointestinal *Candida* colonisation. Concentrations of horseradish peroxidase (HRP) (A) and ovalbumin (OVA) (B) were measured in plasma samples of mice taken at the times indicated after intragastric administration. Values are means (SEM) of six mice per group. * $p < 0.05$ compared with time point = 0; † $p < 0.05$ compared with control mice at each time point.

material dispersed diffusely was taken as evidence of degranulated mast cell. All mast cell counts were performed by a single observer.

Reconstitution of mast cells in W/W^v mice

Mast cell deficient W/W^v mice were reconstituted with in vitro bone marrow derived mast cells (BMMC) from congenic control +/+ mice according to Kung and colleagues.³² Bone marrow from femurs was flushed out and seeded in RPMI1640 medium (GIBCO-BRL, Tokyo, Japan) supplemented with 10 ng/ml recombinant murine interleukin 3 (PeproTech EC, London, UK), 10% heat inactivated fetal calf serum (Biological Industries, Kibbutz Beit Haemek, Israel), 100 U/ml penicillin, 100 μ g/ml streptomycin, and 50 μ g/ml gentamycin, at a concentration of 5×10^5 nucleated cells/ml, and cultured at 37 $^{\circ}$ C in a humidified atmosphere containing 5% CO₂. Culture medium was replaced every seven days. After five weeks of culture, cells were harvested and suspended in PBS. Staining of cells with toluidine blue indicated that nearly 99% of viable cells were mast cells. A total of 1×10^7 BMMC were injected intravenously into each W/W^v mouse through the tail vein. Five weeks later, the reconstituted mice were inoculated with *C. albicans*, as described above, and then subjected to gastrointestinal permeation experiments.

Statistics

Results are presented as means (SEM). The unpaired or paired *t* test or Tukey-Kramer's test following one way analysis of variance was used to compare mean values. StatView for Macintosh (version 5.0, SAS institute Inc., North Carolina, USA) was used for analysis.

RESULTS

Sensitisation against orally administered OVA in BALB/c mice with gastrointestinal *Candida* colonisation

To study the effect of chronic colonisation of *C. albicans* in the gastrointestinal tract on sensitisation against orally administered antigen, BALB/c mice were intragastrically inoculated with *C. albicans*. By weekly counting of faecal specimens after inoculation, a high faecal recovery of *C. albicans* was observed in all mice throughout the experimental period (fig 1A). Additionally, counting of organisms in gastrointestinal tissues by quantitative culture after euthanasia of animals revealed that colonisation occurred in the stomach, jejunum, ileum, and colon in all mice (5.5 (0.2), 1.8 (0.1), 2.3 (0.1), and 3.3 (0.0) log₁₀ CFU/g tissue, respectively). No organism was detected in faeces or tissues of control mice without inoculation of *C. albicans*. These data suggest that chronic colonisation of *C. albicans* was established in the gastrointestinal tract of mice after single inoculation of *C. albicans*.

Figure 1B and 1C show the time course of changes in the titre of serum antibodies against intragastrically administered OVA. In mice with gastrointestinal *Candida* colonisation, the anti-OVA IgG titre began increasing at three weeks after starting administration of OVA and stabilised at six weeks (fig 1B). The anti-OVA IgG titre tended to be higher in mice with *Candida* colonisation than in control mice without *Candida* colonisation throughout the experimental period, and there was significant difference between the two groups at 4, 5, and 7 weeks after starting OVA administration. Anti-OVA IgE titre began increasing at five weeks after initiating OVA administration (fig 1C). As with the IgG titre, the anti-OVA IgE titre tended to be higher in mice with *Candida* colonisation than in control mice, with a significant difference apparent between the groups nine weeks after initial OVA administration.

Permeation of orally administered proteins in BALB/c mice with gastrointestinal *Candida* colonisation

We examined gastrointestinal permeation of HRP in BALB/c mice with gastrointestinal *Candida* colonisation, because it is

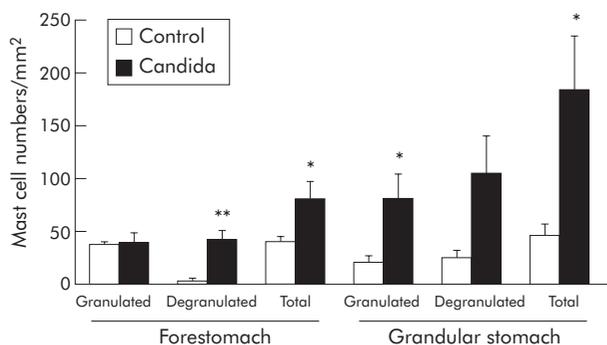


Figure 3 Number of granulated and degranulated mast cells and total number of mast cells in the forestomach and glandular stomach of mice with and without *Candida* inoculation. The cryostat section of stomach was stained with toluidine blue and the number of mast cells in the stomach was counted under high magnification in a section from each sample. Values are means (SEM) of six mice per group. **p*<0.05; ***p*<0.01 compared with control mice without *Candida* inoculation.

simple to detect this macromolecule by ELISA. On the day of the permeation experiment, we confirmed high faecal recovery of *C. albicans* (6.8 (0.2) log₁₀ CFU/g faeces) in all mice inoculated with *C. albicans*. Figure 2A shows the time course of changes in plasma HRP concentrations in BALB/c mice after intragastric administration of this molecule. In control mice without *Candida* colonisation, no significant increase in plasma HRP concentrations was observed after administration. Conversely, plasma HRP concentrations in mice with gastrointestinal *Candida* colonisation increased significantly, peaking at 30 minutes and remaining high for 60 minutes after administration. HRP concentrations were significantly higher in mice with *Candida* colonisation than control mice at 30 and 60 minutes. We next examined permeation of a food antigen, OVA. As shown in fig 2B, compared with control mice, plasma OVA concentrations in mice with gastrointestinal *Candida* colonisation increased significantly 30 minutes after administration.

Mast cell degranulation in gastrointestinal mucosa of BALB/c mice with gastrointestinal *Candida* colonisation

On histochemical examination of gastric mucosa in BALB/c mice killed on the day of the permeation experiment, PAS staining revealed colonisation of *C. albicans* on the surface of the forestomach in all mice inoculated with *C. albicans* (data not shown). Toluidine blue staining showed that a number of mast cells were infiltrated in both the forestomach and glandular stomach in *Candida* inoculated mice, and that degranulation was evident in the majority of cells. Quantitative examination of mast cells in histological sections of stomach in BALB/c mice is shown in fig 3. In the forestomach, numbers of granulated cells were the same in mice with *Candida* colonisation and control mice, whereas numbers of degranulated and total cells (that is, granulated plus degranulated) were significantly higher in mice with *Candida* colonisation than in control mice. In the glandular stomach, numbers of both granulated and degranulated cells were higher in mice with *Candida* colonisation than in control mice.

Permeation of orally administered OVA in mast cell deficient mice with gastrointestinal *Candida* colonisation

To elucidate the involvement of mast cells in increased permeation of OVA in mice with gastrointestinal *Candida* colonisation, we examined OVA permeation in mast cell deficient W/W^v mice. As shown in fig 4A, W/W^v mice with gastrointestinal *Candida* colonisation showed no significant increase in plasma OVA concentrations after administration, and levels were comparable with those in mice without *Candida* colonisation for the duration of the experimental period. Conversely, plasma OVA concentrations increased significantly in congenic littermate control +/+ mice with gastrointestinal *Candida* colonisation. Concentrations peaked at 30 minutes after administration and were significantly higher in mice with *Candida* colonisation than in those without *Candida* colonisation (fig 4B). Quantitative culture of *C. albicans* isolated from pooled faeces for each *Candida* inoculated group showed that the number of organisms was higher in W/W^v mice (6.5 log₁₀ CFU/g faeces) than in +/+ mice (5.5 log₁₀ CFU/g faeces). No organism was detected in the faeces of control mice without inoculation of *C. albicans*. These results indicate that the increase in OVA permeation by gastrointestinal *Candida* colonisation requires mast cells.

To confirm this finding, we then determined whether reconstitution of mast cells enhances OVA permeation in W/W^v mice with gastrointestinal *Candida* colonisation. As shown in fig 4C, BMMC transplanted W/W^v mice with

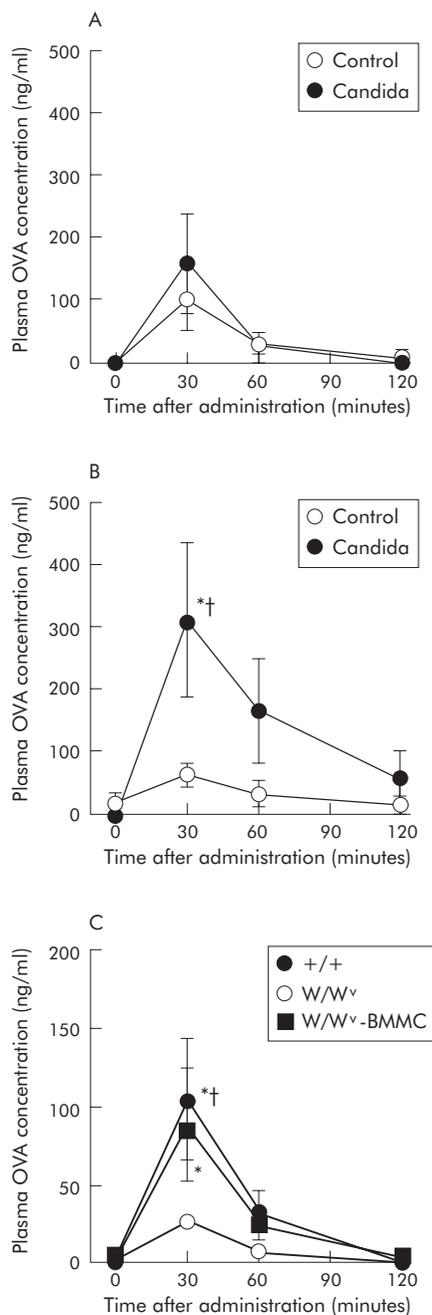


Figure 4 Time course of changes in plasma concentrations of intragastrically administered ovalbumin (OVA) in WBB6F1 mice with and without *Candida* inoculation. OVA concentrations were measured in plasma samples obtained at the times indicated after intragastric administration. (A, B) Results from mast cell deficient (W/W^v) and wild-type ($+/+$) mice, respectively. (C) Data on $+/+$, W/W^v , and bone marrow derived mast cell (BMMC) transplanted W/W^v mice inoculated with *C. albicans*. Values are means (SEM) of six mice per group. * $p < 0.05$ compared with time = 0; † $p < 0.05$ compared with control mice without *Candida* colonisation in (A) and (B) and W/W^v mice in (C), at each time point.

gastrointestinal *Candida* colonisation as well as $+/+$ mice showed a significant increase in plasma OVA concentrations 30 minutes after administration, although no such significant increase was observed in W/W^v mice without BMMC transplantation. OVA concentrations at 30 minutes were significantly higher in $+/+$ mice than in W/W^v mice without BMMC transplantation. In BMMC transplanted W/W^v mice,

OVA concentrations at 30 minutes tended to be higher than in W/W^v mice without BMMC transplantation and were comparable with those in $+/+$ mice. Toluidine blue staining of cryostat sections of forestomach revealed that mast cells were almost absent in W/W^v mice and that there was no significant difference in the number of granulated and degranulated mast cells between $+/+$ and BMMC transplanted W/W^v mice (40 (8) v 42 (7) cells/mm² for granulated cells and 44 (8) v 45 (8) cells/mm² for degranulated cells). In addition, quantitative culture of *C. albicans* isolated from pooled faeces for each group on the day of the permeation experiment showed that the number of organisms was higher in W/W^v mice and BMMC transplanted W/W^v mice (7.0 and 6.8 log₁₀ CFU/g faeces, respectively) than in $+/+$ mice (5.8 log₁₀ CFU/g faeces).

DISCUSSION

The present study showed that gastrointestinal colonisation with *C. albicans* stimulated increased serum IgG and IgE specific to intragastrically administered OVA in BALB/c mice (fig 1), indicating therefore that gastrointestinal *Candida* colonisation promotes sensitisation against food antigen. Theoretically, increased permeation of food antigens in the gastrointestinal tract would elicit such immunological responses as sensitisation. Indeed, intestinal inflammation, associated with increased gut permeability, led to enhancement of sensitisation to ingested milk proteins in guinea pigs.³³ In healthy humans, macromolecules such as food antigens would be excluded by the gastrointestinal mucosal barrier to prevent food allergy. However, in individuals with impaired gastrointestinal mucosal barriers, the likelihood of permeation of food antigens in an intact form would be increased. This is an important prerequisite for the development of a food allergy and may explain the prevalence of food allergy in infants and children with immature gastrointestinal mucosal barriers. In the present study, plasma concentrations of intragastrically administered proteins (that is, HRP and OVA) were significantly increased in BALB/c mice with gastrointestinal *Candida* colonisation (fig 2). This suggests that gastrointestinal *Candida* colonisation impairs the gastrointestinal mucosal barrier, which in turn enhances permeation of food antigens. Consequently, gastrointestinal colonisation by *C. albicans* is likely to promote sensitisation against food antigens, at least in part, by affecting the gastrointestinal mucosal barrier and to increase the risk of food allergy.

We investigated the mechanism by which gastrointestinal *Candida* colonisation facilitates permeation of food antigens through the mucosal barrier of the gastrointestinal tract. Mast cells have been reported to be involved in the regulation of the barrier and transport properties of gastrointestinal epithelium via mediators such as rat mast cell protease II,³⁴ tumour necrosis factor (TNF)- α ,³⁵ and interleukin 4.^{36, 37} In the present study, mice with gastrointestinal *Candida* colonisation showed an increased number of degranulated mast cells in both the forestomach and glandular stomach (fig 3). Under these conditions, mast cells should secrete mediators to increase the permeability of the gastric epithelium. In fact, despite gastric colonisation with *C. albicans*, mast cell deficient W/W^v mice showed no overt increase in the permeation of OVA (fig 4). In addition, reconstitution of mast cells in W/W^v mice with gastrointestinal *Candida* colonisation enhanced OVA permeation whose levels were comparable with those in $+/+$ mice with *Candida* colonisation. The present results therefore suggest that mast cells mediate an increase in the permeation of food antigens in *C. albicans* colonised gastrointestinal tracts. McDermott *et al* reported that mast cells play a critical role in increased intestinal permeability during enteric nematode infection via

release of mouse mast cell protease 1 and degradation of tight junction proteins.³⁸ This may also be the mechanism by which permeation of food antigens in *C. albicans* colonised gastrointestinal tract is increased.

It remains unclear how *C. albicans* colonisation stimulates mast cells. Nosal *et al* reported that cell wall glycoproteins from *C. albicans* induce the release of histamine in isolated rat mast cells.³⁹ In addition, Jouault *et al* showed that phospholipomannan in *C. albicans* stimulated TNF- α production by the mouse macrophage-like cell line J774 via toll-like receptor 2 (TLR2).⁴⁰ Because mast cells reportedly recognise pathogenic products via TLR2,⁴¹ a ligand for TLR2 such as phospholipomannan in *C. albicans* may elicit TNF- α release from mast cells. We also observed that the water exudate of *C. albicans* induces degranulation of rat basophilic leukaemia cells RBL-2H3 in a concentration dependent fashion, suggesting degranulation of mast cells by some water soluble constituents of *C. albicans* (unpublished data). Alternatively, anti-*C. albicans* antibody may stimulate mast cells. We previously observed that the titre of serum IgG1 specific to *C. albicans* increases in BALB/c mice chronically colonised with *C. albicans*.²⁵ Similarly, increased anti-*C. albicans* IgG1 levels in the sera of mice were observed on the day of the gastrointestinal permeation experiment in the present study (data not shown). Although IgE antibodies which bind to high affinity Fc ϵ RI receptors on mast cells play a central role in mediating type I allergic responses, non-IgE antibodies such as IgG1 could also mediate allergic responses via Fc γ RI receptors on mast cells.⁴² Therefore, it seems possible that the *Candida* antigen-IgG1 complex stimulates mast cells via Fc γ RI receptors, which in turn enhances gastrointestinal permeability.

The present study did not elucidate the precise site at which permeation of OVA occurred in mice with gastrointestinal *Candida* colonisation. Although the small intestine is usually considered the primary site of uptake of food antigens,⁴³ previous reports suggest that the stomach is a site of uptake of food antigens under some physiological conditions. Hatz *et al* reported that antigen challenge in rats sensitised with dinitrophenol induces degranulation of mast cells and results in increased permeation of a bystander macromolecule OVA in the stomach.⁴⁴ In addition, gastric *Helicobacter* infection has been reported to increase gastric permeability to macromolecules.⁴⁵ Therefore, it is believed that OVA permeation in mice with gastrointestinal *Candida* colonisation occurred in the stomach where a marked increase in the number of mast cells was observed. Further studies will be required to determine the contribution of the intestines.

Some reports suggest a positive association between *Helicobacter pylori* infection and the development of food allergy^{46,47} and AD.⁴⁸ Matysiak-Budnik *et al* reported that *H. pylori* increases absorption of antigens across the gastric mucosa in mice.⁴⁵ The authors also reported that *Helicobacter* infection inhibits the development of oral tolerance by preventing anti-OVA IgE suppression, normally induced after OVA feeding.⁴⁹ Oral tolerance is characterised by induction of a state of systemic immune non-responsiveness towards antigens present in the gastrointestinal tract.^{50–52} This mechanism presumably prevents the development of food allergy. However, gastrointestinal pathogens, including *H. pylori* and *C. albicans* and/or their products, may disturb oral tolerance induction by affecting the gastrointestinal mucosal barrier and/or by their adjuvant action.⁴⁹ We are currently examining whether gastrointestinal *Candida* colonisation influences induction of oral tolerance in the mouse model.

One may suspect that the bacterial flora in the gastrointestinal tract of *Candida* colonised mice may be involved in gut permeability and antibody responses to oral antigens. In

our preliminary experiments using this *Candida* colonisation model, however, denaturing gradient gel electrophoresis of 16S rDNA amplicons showed that the bacterial composition of the stomach and faecal specimens was the same in mice with and without *Candida* colonisation (unpublished data). Therefore, the possibility described above could be ruled out.

In conclusion, we propose that gastrointestinal *Candida* colonisation promotes sensitisation against food antigens, at least partly due to mast cell mediated hyperpermeability in the gastrointestinal mucosa of mice. In contrast with this murine model however, it is not true to say that *C. albicans* colonises the gastrointestinal tract of a healthy human. *C. albicans* colonises the posterior dorsum of the tongue of 40–60% of healthy humans. Yeast cells dislodge from the tongue, are swallowed, and survive transit through the gastrointestinal tract, being detectable by culture of faeces. In addition, the stomach of humans lacks a non-secretory epithelium which is the major site of *Candida* colonisation in the mouse forestomach. Thus it remains unclear whether the transient yeast cells in the human gut increase the permeability of the intestinal epithelium and/or affect oral immune tolerance in healthy humans. Further studies are required to prove this.

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REFERENCES

- 1 **Verghese A**, Prabhu K, Diamond RD, *et al*. Synchronous bacterial and fungal septicemia. A marker for the critically ill surgical patient. *Am Surg* 1988;**54**:276–83.
- 2 **Bodey GP**. Candidiasis in cancer patients. *Am J Med* 1984;**77**:13–19.
- 3 **Eras P**, Goldstein MJ, Sherlock P. Candida infection of the gastrointestinal tract. *Medicine (Baltimore)* 1972;**51**:367–79.
- 4 **Faergemann J**. Atopic dermatitis and fungi. *Clin Microbiol Rev* 2002;**15**:545–63.
- 5 **Scott BB**, Jenkins D. Gastro-oesophageal candidiasis. *Gut* 1982;**23**:137–9.
- 6 **Lacour M**, Zunder T, Huber R, *et al*. The pathogenetic significance of intestinal *Candida* colonization—a systematic review from an interdisciplinary and environmental medical point of view. *Int J Hyg Environ Health* 2002;**205**:257–68.
- 7 **Nikkels AF**, Pierard GE. Framing the future of antifungals in atopic dermatitis. *Dermatology* 2003;**206**:398–400.
- 8 **Leung DY**, Bieber T. Atopic dermatitis. *Lancet* 2003;**361**:151–60.
- 9 **Eigenmann PA**, Sicherer SH, Borkowski TA, *et al*. Prevalence of IgE-mediated food allergy among children with atopic dermatitis. *Pediatrics* 1998;**101**:E8.
- 10 **Lever R**, MacDonald C, Waugh P, *et al*. Randomised controlled trial of advice on an egg exclusion diet in young children with atopic eczema and sensitivity to eggs. *Pediatr Allergy Immunol* 1998;**9**:13–19.
- 11 **Van Reijssen FC**, Felius A, Wauters EA, *et al*. T-cell reactivity for a peanut-derived epitope in the skin of a young infant with atopic dermatitis. *J Allergy Clin Immunol* 1998;**101**(2 Pt 1):207–9.
- 12 **Jackson PG**, Lessof MH, Baker RW, *et al*. Intestinal permeability in patients with eczema and food allergy. *Lancet* 1981;**317**:1285–6.
- 13 **Majamaa H**, Isolauri E. Evaluation of the gut mucosal barrier: evidence for increased antigen transfer in children with atopic eczema. *J Allergy Clin Immunol* 1996;**97**:985–90.
- 14 **Pike MG**, Heddle RJ, Boulton P, *et al*. Increased intestinal permeability in atopic eczema. *J Invest Dermatol* 1986;**86**:101–4.
- 15 **Farhadi A**, Banan A, Fields J, *et al*. Intestinal barrier: an interface between health and disease. *J Gastroenterol Hepatol* 2003;**18**:479–97.
- 16 **Cenci E**, Mencacci A, Spaccapelo R, *et al*. T helper cell type 1 (Th1)- and Th2-like responses are present in mice with gastric candidiasis but protective immunity is associated with Th1 development. *J Infect Dis* 1995;**171**:1279–88.

- 17 **Ekenna O**, Sherertz RJ. Factors affecting colonization and dissemination of *Candida albicans* from the gastrointestinal tract of mice. *Infect Immun* 1987;**55**:1558–63.
- 18 **Helstrom PB**, Balish E. Effect of oral tetracycline, the microbial flora, and the athymic state on gastrointestinal colonization and infection of BALB/c mice with *Candida albicans*. *Infect Immun* 1979;**23**:764–74.
- 19 **Kennedy MJ**, Volz PA. Effects of various antibiotics on gastrointestinal colonization and dissemination by *Candida albicans*. *J Med Vet Mycol* 1985;**23**:265–73.
- 20 **Mellado E**, Cuenca-Estrella M, Regadera J, et al. Sustained gastrointestinal colonization and systemic dissemination by *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* in adult mice. *Diagn Microbiol Infect Dis* 2000;**38**:21–8.
- 21 **Wiesner SM**, Jechorek RP, Garni RM, et al. Gastrointestinal colonization by *Candida albicans* mutant strains in antibiotic-treated mice. *Clin Diagn Lab Immunol* 2001;**8**:192–5.
- 22 **de Repentigny L**, Phaneuf M, Mathieu LG. Gastrointestinal colonization and systemic dissemination by *Candida albicans* and *Candida tropicalis* in intact and immunocompromised mice. *Infect Immun* 1992;**60**:4907–14.
- 23 **Field LH**, Pope LM, Cole GT, et al. Persistence and spread of *Candida albicans* after intragastric inoculation of infant mice. *Infect Immun* 1981;**31**:783–91.
- 24 **Kennedy MJ**, Volz PA. Ecology of *Candida albicans* gut colonization: inhibition of *Candida* adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. *Infect Immun* 1985;**49**:654–63.
- 25 **Yamaguchi N**, Sonoyama K, Kikuchi H, et al. Gastric colonization of *Candida albicans* differs in mice fed commercial and purified diets. *J Nutr* 2005;**135**:109–15.
- 26 **Marshall JS**, McCurdy JD, Olynych T. Toll-like receptor-mediated activation of mast cells: implications for allergic disease? *Int Arch Allergy Immunol* 2003;**132**:87–97.
- 27 **Yu LC**, Perdue MH. Role of mast cells in intestinal mucosal function: studies in models of hypersensitivity and stress. *Immunol Rev* 2001;**179**:61–73.
- 28 **Reeves PG**, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993;**123**:1939–51.
- 29 **Wang Q**, Fang CH, Hasselgren PO. Intestinal permeability is reduced and IL-10 levels are increased in septic IL-6 knockout mice. *Am J Physiol Regulatory Integrative Comp Physiol* 2001;**281**:R1013–23.
- 30 **Saitoh R**, Yamaguchi N, Sonoyama K, et al. Effect of enzymatic modification of dietary wheat flour for reducing its allergenicity on oral sensitization to and intestinal absorption of ovalbumin. *Biosci Biotechnol Biochem* 2003;**67**:2483–5.
- 31 **Nakajima S**, Krishnan B, Ota H, et al. Mast cell involvement in gastritis with or without *Helicobacter pylori* infection. *Gastroenterology* 1997;**113**:746–54.
- 32 **Kung TT**, Stelts D, Zurcher JA, et al. Mast cells modulate allergic pulmonary eosinophilia in mice. *Am J Respir Cell Mol Biol* 1995;**12**:404–9.
- 33 **Fargeas MJ**, Theodorou V, More J, et al. Boosted systemic immune and local responsiveness after intestinal inflammation in orally sensitized guinea pigs. *Gastroenterology* 1995;**109**:53–62.
- 34 **Scudamore CL**, Thornton EM, McMillan L, et al. Release of the mucosal mast cell granule chymase, rat mast cell protease-II, during anaphylaxis is associated with the rapid development of paracellular permeability to macromolecules in rat jejunum. *J Exp Med* 1995;**182**:1871–81.
- 35 **Mullin JM**, Snock KV. Effect of tumor necrosis factor on epithelial tight junctions and transepithelial permeability. *Cancer Res* 1990;**50**:2172–6.
- 36 **Berlin MC**, Yang PC, Ciok L, et al. Role for IL-4 in macromolecular transport across human intestinal epithelium. *Am J Physiol* 1999;**276**(5 Pt 1):C1046–52.
- 37 **Colgan SP**, Resnick MB, Parkos CA, et al. IL-4 directly modulates function of a model human intestinal epithelium. *J Immunol* 1994;**153**:2122–9.
- 38 **McDermott JR**, Bartram RE, Knight PA, et al. Mast cells disrupt epithelial barrier function during enteric nematode infection. *Proc Natl Acad Sci USA* 2003;**100**:7761–6.
- 39 **Nosal R**, Novotny J, Sikl D. The effect of glycoprotein from *Candida albicans* on isolated rat mast cells. *Toxicol* 1974;**12**:103–8.
- 40 **Jouault T**, Iyata-Ombetta S, Takeuchi O, et al. *Candida albicans* phospholipomannan is sensed through toll-like receptors. *J Infect Dis* 2003;**188**:165–72.
- 41 **Varadaradjalou S**, Feger F, Thieblemont N, et al. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. *Eur J Immunol* 2003;**33**:899–906.
- 42 **Okayama Y**, Kirshenbaum AS, Metcalfe DD. Expression of a functional high-affinity IgG receptor, FcγRI, on human mast cells: Up-regulation by IFN-γ. *J Immunol* 2000;**164**:4332–9.
- 43 **Walker WA**, Isselbacher KJ, Bloch KJ. Intestinal uptake of macromolecules: effect of oral immunization. *Science* 1972;**177**:608–10.
- 44 **Hatz RA**, Bloch KJ, Harmatz PR, et al. Divalent hapten-induced intestinal anaphylaxis in the mouse enhances macromolecular uptake from the stomach. *Gastroenterology* 1990;**98**:894–900.
- 45 **Matysiak-Budnik T**, Hashimoto K, Heyman M, et al. Antral gastric permeability to antigens in mice is altered by infection with *Helicobacter felis*. *Eur J Gastroenterol Hepatol* 1999;**11**:1371–7.
- 46 **Corrado G**, Luzzi I, Lucarelli S, et al. Positive association between *Helicobacter pylori* infection and food allergy in children. *Scand J Gastroenterol* 1998;**33**:1135–9.
- 47 **Figura N**, Perrone A, Gennari C, et al. Food allergy and *Helicobacter pylori* infection. *Ital J Gastroenterol Hepatol* 1999;**31**:186–91.
- 48 **Murakami K**, Fujioka T, Nishizono A, et al. Atopic dermatitis successfully treated by eradication of *Helicobacter pylori*. *J Gastroenterol* 1996;**31**:77–82.
- 49 **Matysiak-Budnik T**, van Niel G, Megraud F, et al. Gastric *Helicobacter* infection inhibits development of oral tolerance to food antigens in mice. *Infect Immun* 2003;**71**:5219–24.
- 50 **Weiner HL**. Oral tolerance: immune mechanisms and treatment of autoimmune diseases. *Immunol Today* 1997;**18**:335–43.
- 51 **Strobel S**, Mowat AM. Immune responses to dietary antigens: oral tolerance. *Immunol Today* 1988;**9**:173–81.
- 52 **Brandtzaeg P**. Mechanisms of gastrointestinal reactions to food. *Environ Toxicol Pharmacol* 1997;**4**:9–24.