

Cow's milk protein induced changes in the expression of HLA-DR antigens on colonic epithelial cells

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Background: Normal colonic epithelial cells do not express HLA-DR antigens unless they become inflamed. It is possible that colonic epithelial cells may function as antigen-presenting cells once HLA-DR is induced by infection or inflammation.

Objective: The aim of this study was to investigate whether cow's milk protein (CMP) is capable of inducing changes in HLA-DR expression on colonic epithelial cells, providing indirect evidence that CMP may activate cell-mediated immune mechanisms within intestinal mucosa.

Methods: HLA-DR expression was evaluated by flow cytometry on cultured human colonic epithelial cell line (HT-29) before and after treatment with bacterial lipopolysaccharide (LPS) or recombinant γ interferon (IFN- γ). Untreated and LPS- or IFN- γ -treated HT-29 cells were then cultured in the presence of CMP. The changes in epithelial HLA-DR expression induced by CMP on untreated and LPS- or IFN- γ -treated HT-29 cells were examined.

Results: Untreated HT-29 cells expressed very little HLA-DR molecule. Bacterial LPS or IFN- γ induced a significant HLA-DR expression on HT-29 cells. When untreated HT-29 cells were cultured in the presence of CMP, there was little induction of HLA-DR expression. Culture of LPS- or IFN- γ -treated HT-29 cells in the presence of CMP induced a significant increase in HLA-DR expression, which was much greater than on HT-29 cells treated with bacterial LPS or IFN- γ only.

Conclusions: Our results suggest that CMP initiates an immune response in the intestinal mucosa and may be responsible for the activation of cell-mediated immunity after enteric infection or inflammation.

Ann Allergy Asthma Immunol 2003;90:348–350.

INTRODUCTION

The characteristic histologic intestinal findings of food protein-induced enteropathy may develop in very young infants with protracted diarrhea after infectious enteritis.^{1–3} There is increasing evidence that local cell-mediated immune mechanism play a role in the pathogenesis of this form of cow's milk allergy. For the initiation of a specific immune response by T cells, antigen must be presented to them by a cell expressing major histocompatibility complex class II molecules. Normal colonic epithelium does not express HLA-DR molecules unless it becomes inflamed.^{4–6} It is possible that colonic epithelium may function as antigen-presenting cell once HLA-DR is induced by infection or inflammation. In the present study, we examined the expression of HLA-DR molecules on the colonic epithelial cells treated with bacterial lipopolysaccharide (LPS) and recombinant γ interferon (IFN- γ). We also investigated whether cow's milk protein (CMP) can induce changes in the expression of HLA-DR on LPS- and IFN- γ -stimulated colonic epithelial cells and might

thereby activate cell-mediated immune mechanisms within the intestinal mucosa.

METHODS

Treatment of Human Colonic Epithelial Cells

The human colonic epithelial cell line (HT-29, obtained from American Type Culture Collection, Rockville, MD) was used as a model of human intestinal epithelium. HT-29 cells were cultured at 37° C in RPMI-1640 containing 10% fetal bovine serum (Gibco, Grand Island, NY). Twenty-four hours after seeding 3×10^5 cells in 60-mL culture dish, 10 μ g/mL LPS (from *Escherichia coli* O111:B4, Sigma, St. Louis, MO) was added in the medium and incubated for 12 hours. Treatments with 10 U/mL, 20 U/mL, and 100 U/mL IFN- γ (Sigma) were performed for 72 hours. Untreated or LPS- and IFN- γ -treated HT-29 cells were washed with 37° C warmed phosphate-buffered saline (PBS) (Gibco) and then cultured in the presence of CMP which comprised 200 μ g/mL of β -lactoglobulin, β -lactalbumin, and α -casein (total 600 μ g/mL, Sigma) for 72 hours. Viability of the cells was evaluated by trypan blue exclusion and was >90% through experiments.

Immunofluorescence and Cell Staining

HT-29 cells were harvested by treatment with 0.2 mg/mL trypsin-ethylenediamine-tetraacetic acid (Gibco) and collected in a 15-mL centrifuge tube. Cells were washed with RPMI containing 10% fetal bovine serum and then PBS. After washing, aliquots (100 μ L) containing 1×10^6 cell

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Received for publication August 29, 2002.

Accepted for publication in revised form November 24, 2002.

suspension were incubated with 20 μ L monoclonal antibodies (HLA-DR-phycoerythrin, Immunotech, Luminy, France) in the dark on ice water for 50 minutes. Unbound antibody was removed by two-times wash with cold PBS. Flow cytometry and analysis of stained cells was performed on EPICS-Elite (Beckman Coulter, Miami, FL).

Statistics

Differences between measurements were evaluated by Wilcoxon signed-rank test. Data are expressed as means $\pm$ standard error of mean. Statistical significance was taken as $P < 0.05$.

RESULTS

Induction of HLA-DR on HT-29 Cells by LPS and IFN- γ

The untreated HT-29 cells expressed very little HLA-DR antigen. Treatment with LPS induced some HLA-DR expression and the percentage of HT-29 cells bearing HLA-DR was increased to $2.9 \pm 1.5\%$. IFN- γ induced HLA-DR expression in a dose-dependent fashion, and the percentage of HT-29 cells bearing HLA-DR was increased to $47.4 \pm 2.3\%$ at 10 U/mL concentration, $56.8 \pm 2.7\%$ at 20 U/mL, and $77.6 \pm 3.6\%$ at 100 U/mL concentration.

The Changes in HLA-DR Expression Induced by CMP

Culture of the untreated HT-29 cells in the presence of CMP induced little HLA-DR expression, and the percentage of HT-29 cells bearing HLA-DR was $1.6 \pm 0.2\%$. Culture of LPS-treated HT-29 cells in the presence of CMP induced significant increase in HLA-DR expression, which was much greater than on HT-29 cells treated with bacterial LPS only (from $2.9 \pm 1.5\%$ to $23.9 \pm 6.2\%$, $P < 0.001$, Fig 1). Culture of IFN- γ -treated HT-29 cells in the presence of CMP induced significant increase in HLA-DR expression, which was much greater than on HT-29 cells treated with IFN- γ only (from $47.4 \pm 2.3\%$ to $79.1 \pm 1.9\%$ with 10 U/mL concentration and from $56.8 \pm 2.7\%$ to $80.2 \pm 2.2\%$ with 20 U/mL concentration, respectively, $P < 0.001$, Fig 1).

DISCUSSION

Major histocompatibility complex class II molecules are usually present on antigen-presenting cells of the immune system, namely dendritic cells, B-cells, monocytes, and macrophages. In the normal small intestine, HLA-DR is expressed by the villous epithelium, only on those epithelial cells involved in nutrient transport from the gut lumen.⁷⁻⁹ Their restricted distribution within the gut epithelium raises the possibility that HLA-DR-positive epithelial cells may function as antigen-presenting cells at the mucosal portal of antigen entry.¹⁰ In human colon, normal epithelial cells have been known to express no HLA-DR molecules. Epithelial HLA-DR expression occurs in acute infective colitis, radiation injury, and chronic inflammatory bowel diseases.¹¹ Thus, it is possible that colonic epithelial cells may function as antigen-presenting cells once HLA-DR is induced. Although a cell line may have limitation that it is not physiologic, HT-29 cells have been used to study the in vitro induction of HLA-DR

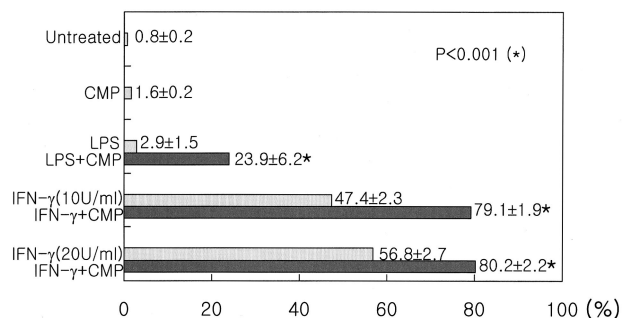


Figure 1. Culture of LPS- or IFN- γ -treated HT-29 cells in the presence of CMP induced a significant increase in HLA-DR expression, which was greater than on HT-29 cells treated with bacterial LPS or IFN- γ only ($P < 0.001$).

molecules on colonic epithelium.¹²⁻¹⁶ In our study, untreated HT-29 cells express very little HLA-DR molecules as shown in another study.¹³ When stimulated with bacterial LPS or IFN- γ , a significant induction of HLA-DR expression on HT-29 cells was observed, which was consistent with the results of the previous studies.^{12,13,16} When untreated HT-29 cells were cultured in the presence of CMP, there was little induction of HLA-DR expression. However, culture of LPS- or IFN- γ -treated HT-29 cells in the presence of CMP showed a significant increase in HLA-DR expression, which was much greater than on HT-29 cells treated with bacterial LPS or IFN- γ only. These changes were evaluated to suggest indirect evidence that a local cell-mediated immune response to CMP was activated after intestinal infection or inflammation.

The postenteritis diet protein sensitivity has been reported in very young infants who presented with diarrhea persisting after enteric infection or intestinal injury.¹⁷⁻¹⁹ Kleinman¹⁹ showed a model of experimental allergic enteropathy that under pathophysiologic conditions, a protein antigen such as ovalbumin may escape the disrupted gastric mucosal barrier and enter systemic circulation with at least some of its epitopes. The physiologic significance of such macromolecular transport may include the recognition of protein antigens by the immune system and induce a transient, reversible enteropathy. The increased epithelial HLA-DR expression during intestinal infection or inflammation, presumably induced by soluble inflammatory mediators, may play a role in the presentation of diet protein antigen. Bland and Warren¹⁰ have shown the HLA-DR-restricted, nonspecific antigen binding by intestinal epithelium. Our results showed that CMP itself induced a significant increase of HLA-DR molecules on colonic cell line pretreated with LPS or IFN- γ . It suggests that a food protein such as CMP can induce a significant increase of HLA-DR antigens on inflamed colonic mucosa which could initiate a cell-mediated immune reaction. The culture of HT-29 cells with other food protein or non-food protein needs to be performed to see whether other protein antigens may have the same effect on colonic mucosa. A previous study has demonstrated that a proinflammatory

cytokine, tumor necrosis factor (TNF)- α , is implicated in the pathogenesis of intestinal dysfunction associated with cow's milk allergy.²⁰ It was reported that TNF- α altered the intestinal barrier capacity, and its effect was enhanced by IFN- γ . We think another study is needed to investigate whether CMP could induce a change in the expression of HLA-DR antigens on colonic epithelium stimulated with TNF- α .

Our study has a limitation that we could not determine in our study whether CMP antigen peptides are actually presented to the T cells in these upregulated HLA-DR molecules. The interaction between HLA-DR-positive gut epithelial cells and the T cells either in the lamina propria or intraepithelial lymphocyte (IEL) population has been demonstrated in several studies. Hoang et al²¹ showed IFN- γ -treated HT-29 cells activated allogeneic IELs in proportion to their expression of HLA-DR molecules. There were controversial findings about whether HLA-DR positive epithelial cells could be implicated in the local activation of CD4+ T helper cells or CD8+ cytotoxic/suppressor cells.²²⁻²⁴ The role of T cells in the lamina propria or IELs in the development of postenteritis diet protein hypersensitivity remains to be confirmed.

CONCLUSION

The present study showed that CMP could induce significant increase in HLA-DR expression on bacterial LPS- or IFN- γ -treated HT-29 cells. Our results suggest that CMP is able to initiate an immune response and may be responsible for the activation of cell-mediated immunity in the intestinal mucosa after enteric infection or inflammation.

ACKNOWLEDGMENT

We thank Shung Chull Chae, MD, PhD for his helpful discussion and critical reading of the manuscript.

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