The immunomodulating properties of HEMA is mediated via formation of the NALP3 inflammasome

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INTRODUCTION

In Sweden as well as in many other countries most materials currently used for dental restorations contain acrylate and methacrylate monomers (1). As a consequence of increased use of resin-based compounds, there has been an increase in reported cases of irritant and allergic contact dermatitis mainly among dental professionals (2-5). The most common agent causing dermatitis among dental professionals is the acrylate monomer, 2-hydroxyethyl methacrylate (HEMA). Individuals in a dental clinic are, not just exposed to HEMA monomers by skin contact, but they are also exposed to HEMA monomers in the air (5).

We have in previous studies shown that HEMA has adjuvant properties. Mice immunized with ovalbumin (OVA) in a solution containing HEMA developed higher anti-OVA IgG1 activity than mice immunized with OVA in a solution without HEMA (6). Mice injected with HEMA-conjugated mouse serum albumin (MSA) produced IgG and IgE autoantibodies (7), and the effect of HEMA on anti-MSA IgG and IgE autoantibody production was dependent on the degree of conjugation of HEMA to MSA. We have also shown that animals immunized with OVA + HEMA have a higher anti-OVA IgE response, which is of special interest with regards to allergy (6). Another finding is that the effect of HEMA is dependent on the buffer used as solvent i.e dissolving HEMA in 100 mM sodium bicarbonate buffer (NaHCO3) results in a stronger anti-OVA IgE response than if HEMA is dissolved in 25 mM NaHCO3 buffer(6).

Toll-like receptors (TLRs) are important components of the innate immune system and belongs to the pattern recognition receptors (PRRs. There are 11 different types of TLRs and each one recognizes a specific type of pathogen-associated molecular patterns (PAMPs). TLR4 at the cell surface of monocytes/macrophages recognize lipopolysaccharides (LPS)(8).

Another member of the group PRRs are nucleotide-binding oligomerization domain receptors, NOD-like receptor (NLR). These are intracellular that recognize patterns of various pathogens and danger signals (8).

There are different subfamilies of NLR and one of them has a Pyrindomain at their amino termini and are known as NLRP. There are a total of 14 different NLR proteins with a pyrindomain, however, the most described one is NALP3 (also called NLRP3 or Cryopyrin) and has the ability to recognize cell damage/stress. In stressed cells, NALP3 interacts with the protease Caspase 1 and an adaptor protein to form a complex called an inflammasome (8).

In healthy cells the NALP3 is bound to accessory protein that keeps NALP3 in an inactive state. There are a variety of stimuli that activates NALP3, and it is believed to be a common factor that triggers i.e efflux of cytoplasmic K+ ions that occurs in stressed cells (9-11). The low intracellular K+ concentration, cause dissociation of the proteins that keeps NALP3 in an inactive state, and thereby Pro-caspase will be cleaved and converted to its active form (Caspase-1), these to react with an adaptor protein and form an inflammasome (8). By
increasing the extracellular concentration of potassium chloride the intracellular decrease in potassium is inhibited and the formation of the NALP3 inflammasome and production of IL-1β is prevented.

The aim of this study was to investigate if HEMA has its effects on the immune system through inflammasome-mediated Caspase-1 activation.

MATERIALS AND METHODS

Reagents and materials
HEMA and Tween-20, Hydrogen peroxide, Sodium phosphate dibasic anhydrous, Trypsan blue solution (0,4%), Albumin from bovine serum, 3,3',5,5'-Tetramethyl benzidine were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Ficoll–Paque Plus from GE Healthcare Bio-Sciences (Uppsala, Sweden). RPMI Medium 1640 (1X) L-Glutamine, heat-inactivated fetal bovine serum (FBS), Dulbecco’s PBS (1x) (without Ca & Mg) sterile and penicillin/streptomycin solution were purchased from Invitrogen (Lidingö, Sweden). Dimethyl Sulfoxide was obtained from Serva Electrophoresis (Heidelberg, Germany). Concanavalin A (ConA) was obtained from MP Biomedicals (Illkirch, France). Sodium Chloride, Potassium chloride and Potassium dihydrogen phosphate were purchased from MERCK (Whitehouse station, USA). Nunc Microwell 96-Well Microplates were purchased from Thermo SCIENTIFIC (Roskilde, Denmark)

Isolation of mononuclear cells from Human Blood
Fresh blood cells supplied by healthy blood donors were obtained from Sahlgrenska University Hospital in Gothenburg, Sweden. Peripheral blood mononuclear cells were isolated by centrifugation over Ficoll-Paque Plus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). The cells were re-suspended in PBS (added Penicillin-Streptomycin) Invitrogen (Lidingö, Sweden), centrifuged and then re-suspended in Dulbecco’s Modified Eagle’s Medium (D-MEM) Glutamax-I supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen AB) (GIBCO®, Invitrogen AB, Lidingö, Sweden). Cell viability was determined by mixing 15 μl of the cell suspension with 15 μl Trypan Blue 0.4%, Sigma-Aldrich (Sweden AB, Stockholm, Sweden) and counted using a Bürker chamber.

THP-1 human monocytic cells
Human monocytic cells were purchased from InvivoGen (San Diego, California, USA). There were 3 different cell lines, THP1-Null cells (positive control cell line to study inflammasome activation as they express high levels of NLRP3, ASC and pro-caspase-1), THP1-defASC cells (with negligible levels of ASC but express native levels of NLRP3 and pro-caspase-1 to determine whether a signal activates the inflammasome and in particular the NLRP3 inflammasome.) and at last THP1-defNLRP3 cells (with reduced NLRP3 activity to study the involvement of NLRP3 in response to a given signal). Cell viability was determined by mixing 15 ul of the cell suspension with 15 ul Trypan Blue 0.4%, Sigma-Aldrich (Sweden AB, Stockholm, Sweden) and counted using a Bürker chamber.

Pre-treatment with Lipopolysaccharides (LPS)
THP1-Null cell suspension were prepared at 2.0x106 cells/ml and 180 ml of cell suspension were added per well. THP1-Null cells were treated with 20 ml of LPS at 10 mg/ml (suspended in PBS) for 45 minutes at 37°C in 5% CO2 (for mononuclear cells from human blood, there was a control group treated with only PBS). Medium was gently removed and supplemented RPMI and HEMA were added,
Treatment with 2-Hydroxyethylmethacrylate (HEMA)
Prior to use, the inhibitor monomethyl-ether-hydroquinone was removed from HEMA by passing HEMA through a column of polystyrene-co-divinylbenzene beads (Sigma-Aldrich, Steinheim, Germany).

Cells were exposed to different concentrations of HEMA (15 μmol/L, 50 μmol/L, 200 μmol/L and 1000 μmol/L), incubated over 1, 3 and 6 days (37°C humidified atmosphere, 5% CO2).

For the mononuclear cells from human blood, a Tissue Culture Plate 24-well Flat Bottom from Sarstedt (Helsinborg, Sweden) were used. To determine and compare whether HEMA activates the NLRP3 inflammasome, Potassium chloride dissolved in medium were added to a control group to block the NLRP3 inflammasome activation.

Enzyme-linked immunosorbent assay (ELISA)
A DuoSet ELISA Development kit from R&D Systems (Abingdon, UK) were used to measure natural and Recombinant Human IL-1 beta according to the manufacturer’s instructions.

RESULTS

Effects of HEMA exposure on mononuclear cells from human blood
Fig 1. Cytokine production in human leucocyte cultures exposed to HEMA for 24 hours. There is an increased production of IL-1β and IL-18 while IL-10 production is unaffected or reduced. IL-1 and IL-18 are cytokine produce after formation of the intracellular NALP3 inflammasome. The figures shows the production of cytokines from one individual. The cytokines where measured in the same sample with a multiplex machine.
Effects of HEMA exposure on mononuclear cells from human blood blocked with Potassium chloride

Figure 2. Human mononuclear white blood cells were enriched and exposed in vitro to HEMA. HEMA exposure induced production of IL-1β. If the extracellular concentration of potassium chloride (HEMA exposure + KCl) was increased, the production of IL-1β was inhibited. Increased extracellular KCl concentration inhibits formation of the NALP3 inflammasome and thus the cells cannot produce IL-1β. Thus the data supports that HEMA exerts its actions on human white blood cells by causing the formation of the NALP3 inflammasome.
Effects of HEMA exposure on THP-1 human monocytic cells

Figure 3. The human monocyte cell line THP1. A variant of the THP1 cell line has a defect in the ability form the NALP3 inflammasome. The wild type THP1 produce more IL-1β (grey) than the wild type cells exposed to saline (blue). The inflammasome deficient THP1 cell line do not produce IL-1β in either case. Hence HEMA induce production of IL-1β via formation of the NALP3 inflammasome.

DISCUSSION

HEMA is a small water-soluble methacrylate monomer found HEMA frequently in dental adhesives in order to increase their wettability and hydrophilicity (11). However, this monomer is often reported to cause skin problems (12). We have in previous studies shown that HEMA has adjuvant properties. Mice immunized with ovalbumin (OVA) in a solution
containing HEMA developed higher anti-OVA IgG1 activity than mice immunized with OVA in a solution without HEMA (6). Mice injected with HEMA-conjugated mouse serum albumin (MSA) produced IgG and IgE autoantibodies (7), and the effect of HEMA on anti-MSA IgG and IgE autoantibody production was dependent on the degree of conjugation of HEMA to MSA.

We have also shown that animals immunized with OVA + HEMA have a higher anti-OVA IgE response, which is of special interest with regards to allergy (6). In continuation of these studies we wanted to investigate the interactions of HEMA on the immune system and if HEMA has its effects on the immune system through inflammasome-mediated Caspase-1 activation.

We cultured mononuclear cells from human blood, pre-incubated them with LPS and exposed them to various concentrations of HEMA (0,15,50,200 and 1000 μmol/ml) and incubated them for 1, 3 and 6 days in vitro. We also had a control group that were exposed to Potassium chloride to increase the extracellular potassium concentration i.e inhibit efflux of cytoplasmic K+ ions that activates the Caspase-1 mediated inflammasome. The results were measured with a DuoSet ELISA Development kit. Increasing the extracellular KCl concentration inhibited production of IL-1β (figure 2).

We did a similar study on the THP-1 human monocytic cell line, where we had 2 different cell lines pre-incubated with LPS and exposed to different concentrations of HEMA (0,15,50,200 and 1000 μmol/L) and incubated them for 1.3 and 6 days. We saw a significant difference in IL-1β production from THP1-Null cells compared to THP1-defASC cells (this variant of THP1 is unable to form a NALP3 inflammasome) when exposed to HEMA. The THP1-Null cells incubated for 6 days with 1000 μmol/L HEMA had 9.4 times higher concentration of IL-1B compared to the THP1-defNLRP3 cells, and 6 times higher concentration then THP1-defASC cells.

DISCUSSION

We have in the present study shown that the effect of HEMA on the human immune system is exerted by inducing formation of the NALP3 inflammasome.

REFERENCES


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MEDELVÄRDET som jag har räknat ut till celllinjerna (t.ex. 3 dygn så var IL-1B koncentrationen 4,8 gånger högre jämfört med NALP3-cellerna). Jag har räknat ut medelvärdet av de värden vi har i tabellen, jag har dock exkluderat 3 dygn NALP3 HEMA 1000 (ett värde på 30,856 som var väldigt lågt jämfört med de andra som låg på ca 60) och även 3 dygn ASC HEMA 1000 (ett värde på 24,631) som också var väldigt lågt jämfört med de andra.