RESPONSES OF NEWBORNS TO TLR LIGANDS

A newborn loses the immunities received from its mother through the placenta by approximately 4 months of age, after which it is relatively defenseless against infection until one year of age or more. Infants are routinely immunized to protect them during this vulnerable period of time. The long-term purpose of my experiment was to develop more effective but at the same time safe vaccines to give to newborns. My goal was to induce Th-1 type T cells safely. To do so, I stimulated the antigen presenting cells with dead bacteria and molecules that come from them, to identify ones that cause antigen presenting cells to give off the right chemical signals, known as cytokines to the T cells. My results show that stimulation of newborn antigen presenting cells through TLR4 or Listeria bacteria, which stimulate antigen presenting cells through multiple TLRs, induced the production of substantial amounts of IL-6 by cells from newborn mice and were relatively more effective in newborns than adults. These stimuli should be studied further to determine their ability to induce Th-1 type immunity in newborns.

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INTRODUCTION

The goal of my study was to identify ways to deceive the innate immune system into thinking there is an infection present, thereby, promoting the adaptive immune response. This response occurs when the antigen presenting cells – the 'scouts' of the immune system - are alerted by receptors, known as Toll-like receptors (TLRs), which detect bacteria and other types of infectious agents. There are approximately 12 different types of TLRs, and each type detects a different kind of infection. Once the infection has been detected, the scouts produce chemicals called cytokines. These cytokines inform the T-cells what it is they are about to fight. When this occurs, these T-cells begin multiplying; they also receive from the 'scouts' information in the form of cytokines that specifies the types of tools they will need to become Th-1 type T cells and to fight off the infection. Once these thousands of Th-1 type T cells have fought off the infection, most of them die. However, a few are left to reproduce themselves in case the infection re-occurs, and it is these persistent Th-1 T cells that provide immunity protect us against this possibility.

MATERIALS AND METHODS

The first step was to harvest the spleen from adult and newborn (four to eight days of age) mice. The supplies needed were a dissection kit (sterilized scissors and forceps), two 15 ml Eppendorf tubes, and 3 ml of RPMI culture medium in each tube. A mouse board, bucket of ice, 95% ethyl alcohol and gloves were also required. Using the above supplies, the mice were euthanized, placed on a mouse board and sprayed with ethyl alcohol. Using scissors, I made a vertical cut in the skin over the left side of the mouse's abdomen, right below the rib cage, then retracted the skin. Using a clean pair of scissors, I cut the abdominal wall and removed the spleen with forceps using the same scissors. I put the spleen in ice-cold RPMI, then isolated the spleen cells. To do so, I first added 3 ml of RBC Lysis buffer to each Petri dish, then added the spleens and, with a frosted slide mashed the spleens into the RBC Lysis buffer to create a single-cell suspension. After mixing with a pipette, the cell suspension was filtered through a cell strainer into a 50 mL tube. The tube was then filled with RPMI, after which the cells were spun down in a centrifuge tube at 1200 RPM at 4 C for 5 minutes. After aspirating the liquid, the cells were resuspended in 3 mL of RPMI. Cells were then counted in Trypan Blue, diluted to the appropriate concentrations and added to wells of 96-well microtiter plates. Stimuli for different TLRs, Listeria monocytogenes (Lm) bacteria, or medium (unstimulated control) were added to different wells. After 24 hours, the plates were centrifuged, the supernatant medium was removed and assayed for the cytokine IL-6 by ELISA.

RESULTS

Results from the last two experiments were analyzed. In both, we used stimuli for TLR 2+1, TLR2+6, TLR 4,

Table 1. Stimulus and IL-6 results

Expt/Group	Unstim	TLR2+1	TLR2+6	TLR4	TLR7/8	TLR9	Lm
1/Adult	ND	980	607	107	4741	5375	ND
1/Newborn	ND	419	257	2522	2054	2656	ND
2/Adult	*	446	570	*	9533	15,600	2114
2/Newborn	69	1821	923	574	520	1686	2382

* none detected
ND - Not done

TLR 7/8, and TLR 9. In experiment 2, we also used Lm as a stimulus (Lm stimulates cells through TLRs 1, 2, 5, 6 and possibly 9). The amounts of IL-6 (pg/mL) in the culture supernatants are shown in the Table 1.

The results shown for Lm were for cells stimulated with the highest concentration. I also tested two lower concentrations of Lm, and at those lower concentrations the newborn had responses of 109 and 608 pg/mL, while the adult had responses of 73.6 and 360.98 pg/mL, respectively.

DISCUSSION

These results indicate that the TLR ligands most useful in stimulating a newborn's immune system are the TLR4 ligand and Lm. These both induced the production of substantial amounts of IL-6 by spleen cells from neonates and should be studied further. On the other hand, TLR7 & 9 ligands in both experiments induced large amounts of IL-6 production by spleen cells from adults; thus, TLR7 & 9 ligands would be the ones to study further if the goal was to stimulate the adult's innate immune system. Furthermore, I believe that the ability of TLR4 ligands and Listeria to induce different cytokines should be studied to see if the results are similar to those for IL-6. TLR2 ligands also need further experimentation to obtain consistent results.

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