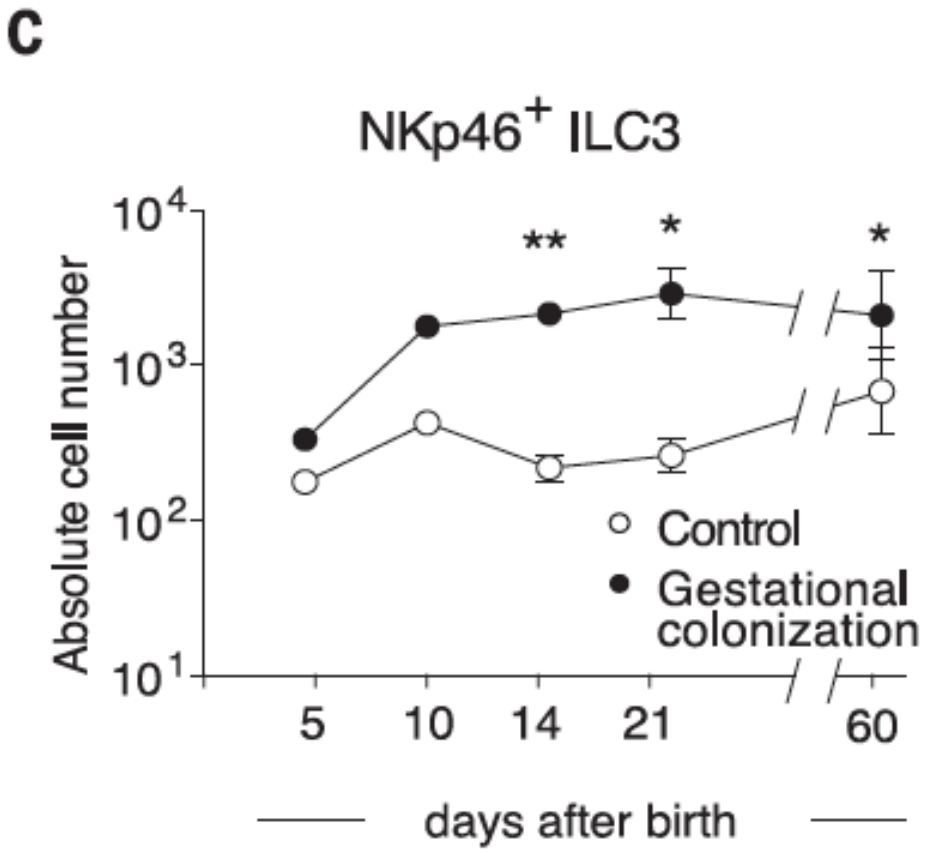
HOW TO USE THIS RESOURCE

Show the figure below to your students along with the caption and background information. The "Interpreting the Graph" and "Discussion Questions" sections provide additional information and suggested questions that you can use to guide a class discussion about the characteristics of the graph and what it shows.



*Caption: The numbers of an innate lymphoid cell, known as NKp46+ ILC3, found in the small intestines of recently born, germ-free mouse pups at various time points after birth. Shaded circles represent pups that gestated inside initially germ-free mothers whose intestines were temporarily exposed to a strain of E. coli during mid-pregnancy (=gestational colonization). Unshaded circles represent controls that gestated inside mothers that remained germ-free throughout pregnancy. Each point represents the mean cell number found in a group of 3-10 pups (+/- one standard deviation). A single star * indicates that the difference between the control and experimental groups has a* p*-value ≤ 0.05 and two stars ** represent a* p*-value ≤ 0.01.*

BACKGROUND INFORMATION

In placental animals, a fetus develops in an almost sterile environment during gestation and typically remains separated from live microbes until birth. After birth, the intestines and other body surfaces become rapidly colonized with a community of largely benign microbes (the microbiota). It has therefore long been assumed that the influence of the microbiota on the development of the immune system does not begin until after birth when newborns come into contact with live microbes in the environment. Scientists challenged this idea by investigating whether microbial colonization of mothers during gestation influences the immune system development of their offspring. Their hypothesis is that bacterial-derived metabolites from the maternal

microbiota reach the fetus (through the placenta) and the newborn (through the milk during lactation), impacting the immune system development of the offspring.

In this study, scientists compared the offspring of two groups of mice, one in which the mothers were kept germ-free throughout pregnancy, and the other in which the mother was germ-free before the pregnancy and was exposed to a strain of *E. coli*, called HA107, in the intestine during mid-pregnancy (gestational colonization). The HA107 strain is engineered to linger in the intestinal tract only temporarily. As a result, the mothers that were exposed to HA107 during pregnancy became germ-free again before their pups were born. To confirm that pups were not exposed to live HA107 or any other bacteria, the scientists tested the mothers' placentas and their newborn pups and found no live bacteria.

By analyzing different types of immune cells populating the intestines of the pups for 60 days after birth, the scientists hoped to discover whether the bacterial exposure of the mothers' intestines during pregnancy influences the development of the pups' immune systems. One of these immune white blood cell (leukocyte) types that they investigated is a subset of the innate lymphoid cell population known as NKp46+ ILC3 (hereafter ILC3). These cells produce and release cytokines, which are small proteins that regulate the function of other immune cells and play an important role in establishing and maintaining the intestinal homeostasis and its relationship with the microbiota. The pups were weaned from their mothers' milk at 25 days, so by sampling for 60 days, they were testing whether the effect on the immune system was persistent.

INTERPRETING THE GRAPH

Between days 5 and 10 after birth, the number of ILC3 cells increased sharply in both groups of pups, but the pups from the mothers exposed to HA107 experienced a steeper increase than pups from unexposed mothers. Between days 10 and 21, pups from exposed mothers experienced an overall increase in the number of ILC3 cells, whereas pups in the control group remained unchanged. At 60 days, the ILC3 cell count remained higher in the pups from the exposed mothers. Based on *p*-values, the differences in ILC3 cell numbers between the two groups were significant at days 14, 21, and 60.

The most striking conclusion from these trends is that in pups born to mothers exposed to the *E. coli* strain during pregnancy, the development of immune-related ILC3 cells in their small intestines during the first 60 days after birth is enhanced compared with pups born to unexposed mothers. This suggests that the mothers' bacterial exposure was enough to elicit an immune response in the pups even without their direct exposure to live bacteria (as confirmed by tests finding no live bacteria in mothers' placentas or pups' intestines). This finding contradicts previous assumptions that the impact of the microbiota in the development of the immune system mainly starts at birth.

Experiments conducted later in this study showed that the mothers' antibodies were required for the enhanced development of the immune system of the offspring. These antibodies could have been passed to the offspring through the placenta during gestation and through the milk after birth. In the mother, these antibodies caused the retention of small metabolites released in the intestine from HA107 during pregnancy. The metabolites were later passed to the pups via the placenta and in the milk. The study shows that the mothers' microbiota and antibodies trigger the growth of ILC3 cells in the intestines of the offspring.

These lines of evidence led the scientists to conclude that the pups' immune system development was due to the transmission of microbial metabolites promoted by the passage of maternal antibodies across the placenta and through the milk during lactation.

Teacher Tip: Prompt your students to explain the following:

- Graph Type: Line Graph
- *Y*-Axis: Number of ILC3 cells found in the small intestines of mouse pups at different times after birth. This is a logarithmic scale as opposed to a linear scale.
- *X*-Axis: Time (number of days after birth)
- Legend: Unshaded circles represent cell counts from the control group of germ-free mouse pups whose mothers were never colonized by HA107. Shaded circles represent cell counts from germ-free mouse pups whose mothers were transiently exposed to HA107.
- Data Types: Each point represents the geometric mean (because of the logarithmic scale) of the absolute cell count found in groups of 3 to 10 mouse pups. Bars represent one standard deviation. (Note, these are not the same as standard error bars.) The *p*-values indicate the probability that the number of ILC3 cells found in the control and experimental groups were due to random chance alone.

DISCUSSION QUESTIONS

1. What trend(s) do you see in ILC3 numbers in the experimental and control groups of mouse pups?
2. Why is it important to show the control group on the graph?
3. Describe what is happening to the variation in ILC3 numbers (represented by standard deviation) over the 60 days. How might a larger sample size affect this variation?
4. If a reasonable null hypothesis is that there is no difference in the number of ILC3 cells between the control and experimental mouse pups, do the *p*-values reject this null? Cite evidence from the graph.
5. How long does the effect from the maternal exposure last in this study? What is your evidence for this claim?
6. How is it possible that the ILC3 trend continued even after weaning at 25 days?
7. Why is it important to the experiment that no live microbes were detected in the placentas of the mothers or the intestines of the newborn pups?
8. How is the increase in ILC3 in the "gestational colonization" group possible without direct exposure to the HA107 bacteria? In other words, can you explain how the mother's immune response might contribute to this effect in her pups?
9. Use evidence from the figure to support the scientists' claim that maternal exposure to bacteria affects postnatal immunity.
10. Based on this study, what can you infer about the impact of the mother's immune system on the pup's future exposure to environmental bacteria? Why is this information useful?

KEY TERMS

bacteria, cytokines, *E. coli*, gestation, immunology, innate lymphoid cell, microbiota, reproduction

SOURCE

Figure 1C:

Mercedes Gomez de Agüero, *et al*. 2016. The maternal microbiota drives early postnatal innate immune development. *Science* 351(6279): 1296-1302.

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