Making flies sick reveals new role for growth factors in immunity

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A Salmonella infection is not a positive experience. However, by infecting the common laboratory fruit fly *Drosophila melanogaster* with a Salmonella strain known for causing humans intestinal grief, researchers in the School of Life Sciences at Arizona State University have shed light on some key cell regulatory processes – with broad implications for understanding embryonic development, immune function and congenital diseases in humans. Associate Professor Stuart Newfeld and laboratory coordinator Joel Frandsen, along with colleagues in the College of Liberal Arts and Sciences and Biodesign Institute at ASU, released their findings online on September 24 in the journal *Proceedings of the National Academy of Sciences*.

Strong parallels exist in the regulation of immune system function in animals as diverse as flies, mice, and humans. Newfeld's own investigative connection between fly and human immune systems came about through his research with a well-studied family of proteins called Bone Morphogenetic Proteins (BMP).

"Bones and flies?" one might scoff. These proteins are named because of their involvement in the formation of bone and cartilage in humans; however, they have also been linked to many other aspects of early development and to essential cellular processes in virtually all animals.

One type of morphogenetic protein, intensively studied in fruit flies and the focus of the published study by the Newfeld group, is the growth factor Decapentaplegic (Dpp). Dpp acts as a hormonal signaling device,

binding to cells and communicating, for instance, whether to divide or to stop growing or even to become a different type of cell.

Studies have shown that Dpp in the fruit fly and its counterparts in other animals have diverged little from one another in evolutionary time. Although there are tiny changes in the genes that code for this protein from animal to animal, the morphogenetic proteins are still structurally and functionally very similar – a testament to their crucial role as signaling devices in all animals, including humans.

"Dpp from flies has been shown to be completely functional in mammalian cells, and the human version of Dpp – BMP 2/4 – also works just fine when injected into flies," explains Newfeld.

Newfeld's research builds on earlier observations made by Aaron Johnson, then a graduate student in the Newfeld lab, now a postdoctoral fellow with University of Texas Southwestern Medical Center in Dallas, Tex. Johnson first observed that fruit fly mutants that lacked the ability to generate Dpp protein in one tissue (at a particular time in embryonic development) suffered from excess cell growth in the neighboring tissue. The lack of communication between the tissues resulted in uncontrolled cell growth, in this case in the heart. "Dpp mutant flies have large hearts which are stiffer and beat inefficiently," says Newfeld.

Johnson went on to uncover Dpp's role in heart development. He discovered that when the embryo is nearly ready to hatch, Dpp signals tell heart cells to stop growing. These instructions also insure a proper boundary between the heart and surrounding muscle tissue.

While these were fundamentally exciting discoveries, Newfeld made them even more so when he extended Johnson's project. Since both the heart and the lymph glands in the fly originate from the same tissue (cardiogenic mesoderm), he postulated that when heart development

goes awry in fruit fly Dpp mutants that the lymph glands might also be affected.

"One of the functions of the lymph gland in fruit flies is to produce blood cells," notes Newfeld. "This is in contrast to humans where the processes take place in our bone marrow."

With support from Science Foundation Arizona, Frandsen built on Johnson and Newfeld's early discoveries by looking into the mutant fruit fly's immune system and blood cells. He noticed that, in addition to excess cell growth during heart development, Dpp mutants also had an excess of plasmatocytes (blood cells involved in digesting small infectious particles) – an important clue that Dpp was affecting the regulation of the immune system of the flies. Plasmatocytes are one of three types of immune cells that arise from hematopoietic stem cells – embryonic cells that only take on their adult roles based on signals they receive from signaling molecules, including Dpp. However, how Dpp might specifically function in blood cell formation remained a mystery, and required fresh thinking.

With help from School of Life Sciences Professor Roy Curtiss, director of the Center for Infectious Diseases and Vaccinology at the Biodesign Institute at ASU, and his technician Bronwyn Gunn, Frandsen and Newfeld developed a novel experimental approach. Rather than curing their fly patients, they sought instead to make the Dpp mutants sick, hoping the infection with Salmonella would provide a new avenue to study their immune system defects in greater detail.

"The problem with the traditional approaches to studying immunity is that we keep our flies in a pretty clean lab – they see few, if any, pesticides or parasites or anything they would need to defend themselves from," says Newfeld.

Getting flies sick wasn't trouble free, Frandsen says. At first, the fruit flies wouldn't eat the type of Salmonella that infects humans. But, with some clever cookery, a feeding technique was identified that led to Salmonella-infected flies. Once inside the flies, the Salmonella activated their immune systems. Newfeld points out that it was then that Frandsen made a key observation: Dpp mutant flies are unable to produce one type of immune cell that normal flies do in response to an attack by pathogens. This was the first piece of evidence that Dpp might regulate the options available to hematopoietic stem cells.

The lack of Dpp in fly mutants meant that their stem cells would only become plasmatocytes. The inability of Dpp mutant fruit flies to produce a particular immune cell type was not obvious under regular lab conditions. This type of defect is considered "cryptic"; a defect that is not immediately obvious because until the fly requires an immune response there is no way to know that something is wrong.

"Up to this point Dpp had not been implicated in hematopoiesis in flies," states Newfeld.

The discovery that Dpp plays a direct role in immune system regulation in flies may have some direct implications for humans, offering new insight into human diseases caused by mutations in bone morphogenetic proteins. Newfeld says too, that scientists who study these morphogenetic proteins in mammals (proteins very similar to Dpp) have known for some time that these proteins are involved in the hematopoietic stem cell growth in the bone marrow. The similarities between the two organisms are intriguing.

"These are exciting parallels; ones which can stimulate collaboration, provide inspiration and reveal new research directions relevant to the understanding of development and immune diseases," Newfeld notes.

Source: Arizona State University