Cell

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Dear Dr. Hoffmann:

Thank you for the manuscript that you recently submitted to Cell. I have enclosed the comments of the reviewers.

You will see that all three of them are generally supportive of the publication of this work in Cell and I am therefore happy to accept the manuscript in principle. However, it is clear that a certain amount of revision will be required. You will see that the referees think that it is necessary for you to provide some further information. I think that these requests for additional experimental data are quite clear and that they do not require any amplification from me.

In revising the manuscript it will be essential for you to ensure that it is within our length limits that are layed out on the enclosed sheet.

If you think that you can respond satisfactorily to the criticisms of the referees, and provide the additional data that they request, then you should let me have four copies of the revised version of the manuscript within two months,

Yours sincerely,

Peter W. J. Rigby

dd/PWJR/X0314E

An

MS X0314

Lemaitre et al. present evidence that the Toll signaling pathway participates in the immune response in Drosophila. Previous studies have shown that an elaborate signaling pathway controls the transport of the maternal dorsal regulatory protein from the cytoplasm to the nucleus of early embryos. Once in the nucleus, dorsal plays a key role in dorsoventral patterning and the differentiation of embryonic tissues. In the present study, the authors present evidence that several components of this dorsal nuclear transport pathway also influence the immune response in adult flies. In particular, evidence is presented that mutations in the Toll receptor, its ligand (spatzle), and cytoplasmic mediators (tube, pelle, and cactus), attenuate the induction of an antifungal gene, drosomycin.

The basic conclusion here, that a common signaling pathway controls both a developmental process (dorsoventral patterning) and a physiological process (immunity), is quite exciting and definitely of sufficient general interest to justify publication in Cell. However, I believe the authors ought to rework the study in order to provide more definitive evidence for a direct link between embryonic patterning and immunity, as discussed below.

First, the authors should determine whether Dif nuclear transport is blocked or attenuated in spz, Toll, tube, pelle, and cact mutants. This information is essential to determine whether the Toll pathway is directly mediating the immune response through a Rel transcription factor. Second, does the drosomycin promoter region contain kB-like recognition sequences? Ideally, the authors would eliminate the induction studies on diptericin and cecropin, which are essentially unaffected by mutations in the Toll pathway. This information could be substituted with drosomycin promoter fusions showing a direct transcriptional response to Toll dominant mutations, and other disruptions in the pathway. Third, the authors discuss separate antibacterial and antifungal components of the immune response, but do not demonstrate this in the Northern assays. Figs. 1-4 present the results of bacterial infections, which is bit odd since drosomycin is an antifungal gene. Is there an uncoupling of the drosomycin and cecropin responses when flies are injected with Aspergillus? The authors analyze the survival of flies infected with bacteria vs. fungi, but do not correlate this with drosomycin induction in the Northern assays And finally, Fig. 5 should be extended to show hypersensitivty of spz, tube, and pelle mutants to fungal infection. On a related note, are Toll dominant and cact mutants able to fight off fungal infections that are normally lethal due to constitutive expression of drosomycin?

Minor points:

-pg. 3. It's a bit strange to call the fat body a functional homolog of the mammalian liver

-pg. 3. What is meant by "recently isolated a recessive mutation" ? Was the gene cloned?

-pg. 13. beginning of Discussion; and elsewhere throughout the text. The authors have not analyzed "...the transcription of the drosomycin gene.." They have examined the steady-state levels of drosomycin RNA.

-pg. 13. Evidence that Toll and cact are expressed in the fat body should be presented, rather than stated as unpublished.

X0314E Lemaitre *e t al.* **Reviewer No. 3**

This paper shows that many of the genes controlling dorsoventral signalling in *Drosophila* are also required for induction of the antifungal *drosomycin* gene in adult flies. It shows that adults which fail to induce antibacteriocidal proteins are sensitive to bacterial infection, and that *Toll* mutant adults, which are defective in inducing *drosomycin*, are sensitive to fungal infection.

This is an interesting paper, which establishes previous conjectures that related signal cascades control immune responses in *Drosophila* and vertebrates. This result is clearly of general significance for studying and understanding the regulatory pathways and evolution of host-pathogen defence systems. The paper would benefit considerably if written more concisely, but I support publication in *Cell* if the authors deal with the criticisms below.

- The authors should strengthen their data on *drosomycin* expression by showing that it is constitutive in *Tl; cact* flies, i.e. that Toll acts to counteract negative regulation by Cact. Similarly, the sensitivity of *Tl; cact* flies to fungal infections should be tested.

- The authors tested only *Tl* mutations for sensitivity to bacterial and fungal infection and injury. They should have included at least one further member of the pathway, alone or in combination with *imd* as appropriate.

- I was unclear about the justification for stating that components of the DV pathway are also functional in larvae (page 11 para 1). Surely, the mutant analysis shows no role for this pathway in inducing anti bacterial proteins in larvae.

Lemaitre et al (Hoffman)
X0314E
The dorsoventral regulatory gene cassette...

This very interesting manuscript presents the first data showing a functional role for the Drosophila Toll pathway in the Drosophila immune response. The authors provide convincing data that Toll is required in adults for the induction of the antifungal peptide drosomycin. The authors provide clear data that in whole animals Toll mutant flies will die as the result of fungal, but not bacterial, infection. In contrast, mutants in another gene, imd, cause death as the result of bacterial, but not fungal, infection. These whole animal results provide a vivid demonstration of the functional importance of these molecules. Furthermore, the results show that Toll is important in this response in adults, but not in larvae. These results provide considerable light on what had been a confusing set of data that implicated Toll in the immune response without defining its function. The paper is well written, and the introduction is especially well laid out. Because of the evolutionary conservation of the innate immune response, these results are of considerable general interest and are, in principal, important for publication in Cell.

There are, however two significant problems that need to be addressed by the authors prior to publication.

The first problem is ruling out the possibility that the mutants in the Toll pathway affect the immune response indirectly. For instance, it is possible that, rather than the postulated role in signaling, these mutants cause developmental defects or global physiological abnormalities that lead to the observed effects on drosomycin expression and susceptibility to fungal infection. The authors apparently have data using temperature sensitive alleles that could address this point by showing that the temperature-sensitive period occurs shortly before the immune response, but the data is not shown. It is important to show this data for both Toll alleles used: the text reports the effect only for Tl^{r632}, but Tl^{r444} is even more temperature-sensitive and should provide the cleanest data.

The second problem is that the role of the other genes in the pathway, particularly spätzle, in this aspect of the immune response is less convincing. The effect of spätzle mutants shown in Figure 1 on drosomycin expression seems much weaker than the effect of Toll. The authors note other anomalies in the effects of spätzle mutants, such as the weaker effect of spz mutants than Toll, tube and pelle mutants on the inducibility of attacin and defensin (which appears to be no effect at all in spz mutants). As the authors themselves point out on p.14, the involvement of spätzle in the immune response was unexpected since mutants of this gene do not affect viability. Furthermore, in contrast to Toll, which has been shown to be expressed in the fat body, it has not been demonstrated directly that spätzle (or tube or pelle) is even expressed at the relevant stage in the appropriate tissues. A role for spätzle in this pathway would be very exciting, and must be documented more clearly prior to publication.

Minor points:

The data in Figure 2 would be easier to understand if presented as points or lines with error bars, with all genotypes pooled.

The point on p. 15 of the discussion about the two pathways that can trigger drosomycin expression is weak. The differences between adults and larvae alone make it clear that there are two pathways. However the argument that the two pathways work on the same rel protein is unconvincing. The data do argue that activity of a Rel protein is sufficient to activate drosomycin expression, but they do not eliminate the possibility that some other active transcription factor could also be sufficient.

There are large numbers of spelling and grammatical mistakes. The second full paragraph on p.12, for instance, starts with two very poor sentences.