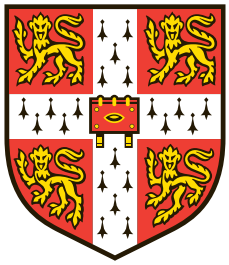
# Immunity Against Malaria: an Atlas of the Mosquito Cellular Immune System at Single-Cell Resolution



**Gianmarco Raddi**

Wellcome Sanger Institute

University of Cambridge

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*Acta est fabula, plaudite*

# Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit for the relevant Degree Committee.

Gianmarco Raddi

September 2019

**Gianmarco Raddi** – Immunity Against Malaria: an Atlas of the Mosquito Cellular Immune System at Single-Cell Resolution

# Abstract

Malaria is a deadly, worldwide disease, yearly responsible for 219 million cases and over four hundred thousand deaths[1]. The *Anopheles gambiae* species complex is the main African vector for the most virulent malaria parasite: *Plasmodium falciparum*[2]. Mosquitos are not mere bystanders however, and rely on both humoral and cellular innate immune divisions to defeat invading pathogens[2, 3]. These efforts are coordinated by hemocytes, the insect equivalent to vertebrate's white blood cells, circulating in the hemolymph within the insects' body cavity. Yet, hemocyte biology is largely unknown, mainly due to the low number and fragility of mosquito immune cells[4]. Here we dissect the *Anopheles* immune system under baseline and challenged conditions with single-cell RNA sequencing to identify previously unknown cell types, their gene signatures, and spatial-temporal localization in the mosquito. We profiled 5,292 individual *Anopheles* hemocytes 1,3 and 7 days after sugar-feeding, blood-feeding, or infection with *Plasmodium berghei,* as well as 3123 *A. aegypti* hemocytes. We identified 9 cell sub-types, including novel effector, phagocytic, and anti-microbial cell subtypes, in addition to dividing progenitor cells, validating the main cell types via correlative microscopy and morphology. And we described four lineages of hemocytes, showing them to be divided into two branches: oenocytoids and prohemocyte-granulocyte. We also found both blood-feeding and malaria infection to dramatically shift the composition of a mosquito's immune system, activating effector and proliferating cells at days 1 and 3 before returning to baseline by day 7. Conversely, human *P. falciparum* appears to inactivate an important local effector subtype. Our work is the first comprehensive transcriptomic study of a whole insect immune system. It demonstrates hemocytes are a dynamic, diverse class of insect cells which complexity far exceeds what is currently described in the literature. Our methods and results will hopefully serve as a resource for many entomologists, and could prove useful in developing novel vector control strategies. Our website will ease data access and provide an intuitive way to visualise hemocyte information: https://hemocytes.cellgeni.sanger.ac.uk/

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walked the immortal city (and you are far from being alone). The word panache has been created to describe you. Eric! Oooooh where to start. Where to end? Should I bring into the picture Tomas? Or the honorary Italian? Or the master chef? Or the closeted Republican? Joking, joking. The only true closeted Republican can be nobody else but the funniest m*********r 'merica has ever created: Anne. Keep being boss darling dear.

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"Looking up at the stars, I know quite well
That, for all they care, I can go to hell,
But on earth indifference is the least
We have to dread from man or beast.

How should we like it were stars to burn
With a passion for us we could not return?
If equal affection cannot be,
Let the more loving one be me.

Admirer as I think I am
Of stars that do not give a damn,
I must not, now I see that, say
I missed one terribly all day.

Were all stars to disappear or die,
I should learn to look at an empty sky
And feel its total dark sublime,
Though this might take me a little time."

W.H. Auden

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