

Research Article

Good news from Alsace: the Covid-19 pandemic is (almost) over. NOW WHAT?

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For the last 4 or so months we have been rushing up to find answers to big questions raised by the Covid-19 pandemic. Besides the obvious ones, relating to the urgency to discover something that could curtail the constant bleeding of deaths caused by the Acute Respiratory Distress Syndrome, there has been a lingering mystery about the disease. This mystery can be formulated as follows: How is it possible that so many highly exposed people, including many with obvious clinical signs of being infected, are persistently negative both in the qRT-PCR and the serum antibody test? A paper posted yesterday by a French team provides a more than reasonable explanation to that conundrum and, by doing it, put us in a position to start thinking in the beginning of the end of this pandemic.

For the last three and a half months, I, as many others I suppose, have been spending my mornings and evenings poring over rims of scientific reports in search of a thread that I could pull and hopefully find the definitive clue. Today, this very morning, after all these months of light sleeps over late night thoughts, I finally found the end of the thread. Sure enough, it came in the form of a preprint.

The paper by Floriane Gallais et al. from Professor Samira Fafi-Kremer Virology lab in Strasbourg, France, posted yesterday 22/02/2020, bears the following cool unassuming title "Intrafamilial Exposure to SARS-CoV-2 Induces Cellular Immune Response without Seroconversion"^[1]. When you hear a hit song for the first time, although still unfamiliar with the melody or the lyrics, that very moment, you know this song is going to take over the charts. This is what I felt after quickly first cross-reading the paper a few hours ago. I did it with excitement because I knew already something about it...

So let's rewind a little bit. It (this chapter of the Covid-19 story) all started for me around three weeks ago. The morning of that day, in the midst of the first moves towards the official deconfinement in Spain, I woke up with a hunch. As I was preparing my breakfast, I kept repeating to myself: "The

pandemic is over. The pandemic is over". "What the hell is going on in my head?" I thought. "Am I going crazy after so much science literature running through my brain circuits?" I asked myself. "Now that everybody is concerned about a second and a possible third wave of the pandemic, or about the dramatic surge of the death toll in South and Central America, I, of all people (much smarter than me) heaving on the subject, come up with such a nonsense?" I reflected. Over the following hours, the thought didn't leave me a second. I was so possessed by it that I was forgetting my mask everywhere and, just like a kid with his Christmas new bicycle, I was telling my colleagues the good news.

"But, how come?" A colleague of mine dared to ask. "The Hidden Immunity" I prompted, without remotely knowing what I was talking about. "What?" he exclaimed in disbelief. "I don't know, it is something like the *Dark Immunity*, I guess" I said. That was all for that day.

Over the ensuing days, I tried to come up with a more coherent and elaborate answer to my colleague's question. I reviewed what I had learned so far about the adaptive immune response to the CoV-2 virus. I knew already that the antibody response in the Covid-19 disease is already unusual in several regards, raising many questions about what, if any, would be the role of humoral immunity in building a protective barrier to future encounters with the virus. I also delved into the uniqueness of the cellular immune response to the virus. Since I am the farthest from being an immunologist, I had to quickly refresh the few notions that I have kept from my graduate times and started dredging through the avalanche of CD+ and CD- with their highs, lows and intermediate expressors. So I finally got some decent update on cellular immunity, just enough to realize that something unique was going on in that territory too.

To summarize a few things that I've learned lately:

Humoral immunity in Covid-19:

1) The IgM, IgG response in Covid-19 is synchronous, meaning that both IgG and IgM often increase simultaneously in the blood of patients a few days after the infection^[2]. This is unlike what happens after a first encounter with any other human viruses, where IgM is first produced and then, approximately one week after, the so-called Ig class switch takes place and IgG takes over, becoming the main Ig being produced and the champion of the long term humoral immunity. Several interpretations have been produced to explain this phenomenon. My favorite one: there have been previous encounters with close coronavirus relatives, the so-called benign coronaviruses causing 10%

of the common colds that we all have suffered through the winter months for the last century or so. This is called cross-immunity or cross-reactivity^[3].

2) In the mild forms of the Covid-19 disease, immunoglobulins being produced have a poor neutralizing activity against the virus^{[4] [5]}. One would expect just the opposite, right? You would think that, in those patients, a good neutralizing antibody response would quickly quench the viral infection resulting in a milder disease. Not the case. This implies that something else besides antibodies would be behind the milder course of the disease.

3) The neutralizing power is higher in anti-CoV-2 antibodies isolated from the more severe patients^{[6] [7] [8]}. Again a paradox? Not really. It could well serve to argue that antibodies are not "the thing" that can protect us against the virus.

Cellular immunity in Covid-19:

1) Lymphopenia (at the expense of T cells) is a hallmark of the disease. Among T cells, CD8+ T cells, the ones supposed to mount a proper response to a virus through the recognition of viral peptides bound to the MHC I on antigen presenting cells, seem to be particularly poorly represented in the more severe presentations of the disease)^{[9] [10]}

2) The balance between T and B cells is tilted towards the latter (particularly in the severe cases). As we have seen, B cells don't seem to be a good investment in this disease^[11].

3) As with antibodies, there is a clear and relatively prominent cellular cross-reactivity with other coronavirus. In other words, thanks to having been previously exposed to some of these "common cold" coronaviruses, most people already have some T cells in their blood with the capacity to cross-react with the CoV2 virus^{[12] [13]}.

So, after sifting over hundreds of papers on the subject, I started thinking that maybe cell immunity, not humoral immunity could be behind the good or bad outcomes of the disease.

This would fit well with what I kept witnessing since almost the beginning of it all: In my closest circles of F&F as well as among many of my pediatric patients, particularly those with textbook clinical features of the disease (including being themselves close contacts of confirmed cases), people kept testing stubbornly negative for both PCR and serum antibodies. Having to handle this kind of negative results used to leave me with a sweat/sour taste in my mouth. On the one hand, I had the opportunity to deliver "good news" to the family: "He/she doesn't have the virus", but, on the other hand, what I

thought was a clear case of Covid-19 now became a puzzle. A puzzle that I had to deal with several times a day for the last couple of months. Until today.

To recapitulate, all of the above have led me to conclude that the whole humoral response (i.e. antibodies) in this disease is probably just a bystander, and that, even in those that mount a good antibody response, it is to the antigen specific T cell defense that we should attribute the good outcome observed in most patients with Covid-19.

The Strasbourg Paper

So let's get to the seminal paper: This is a short humble study dealing with the consistent finding of negative tests (of both CoV-2 qPCR and serum antibodies against the virus) in family members living together with confirmed cases. In addition, in agreement with what many of us had observed during our practice for the last few months, most of these cases presented with mild clinical signs of the disease at the time of or shortly before the tests. So the authors asked a simple question: What if humoral immunity doesn't cut it in these cases and instead it is the cellular branch of the immune system that is pulling the strings? To answer this simple question they went on to isolate the presumed culprits (CD3+ T cells, no distinction between CD4+ or CD8+) from all the subjects in the study and did a simple test on them: They studied their response to overlapping peptides from the major structural proteins of CoV2 (Spike, Nucleocapsid, Membrane, Envelop) and from some non-structural proteins as well. To do that they set up a classic test in immunology whose read out is the production of gamma-interferon by stimulated T cells. And then, Bingo! all the co-habitants of confirmed Covid-19 cases, the ones with negative qPCR and antibody tests, were indisputably positive in the so-called quantiferon test. They tested positive to peptides from some or most of the viral proteins. Importantly, the positive response was also against non-structural proteins, the ones that need intracellular viral infection to be bound by the MHC proteins and then exposed on the surface of APCs to finally be recognized by T cells. The authors conclude that a low titer infection could be the reason for the continuous negative qRTPCR results in these patients, which sounds as a reasonable explanation to me.

To nail down the argument, the authors discuss what I think is a very important precursor of their paper. As they mention, a similar phenomenon has been previously reported on the hepatitis C virus (HCV). Indeed, health workers with a recent proven exposure to HCV persistently had negative viremias as well as antibody tests^{[14] [15]}. Just like here. And just like in our case, they tested positive

in the immunoferon assay against non-structural proteins of HCV. Importantly, the response in [13] lasted for a short average of 12 weeks. Now the question is if Covid-19 behaves the same way and if so, will there be memory cells in the immune system to remember the previous encounter with CoV-2, so we can properly respond should we have a new rendez-vous with the virus? My gut (and scientific) answer to that, you guessed it, is yes.

Obviously the data need to be confirmed on a bigger scale, and, also, using different non-structural proteins from the CoV2 proteome as well as a better defined true negativity in unexposed people. Most importantly, as argued upon above, cell immunity as described in Gallais et al. paper has to be tested for longer periods in order to prove long time protection. Also, more sophisticated models should be used to assess if these reactive cells are really protective against a viral challenge in vitro and in vivo.

What Now?

Although the authors don't adventure to guess it, according to my estimates, by counting the potentially "cell immunized" together with the seropositive population, at least here in Spain as well as in some other European countries, we probably have already reached a decent, if not a robust, herd immunity for the CoV2 virus. Only time and future mass testing will tell us what is the real scope of this immunity coverage. There seems to be an agreement among the experts on that: for a good herd immunity we would need at least 70% of the population to be protected against the infection^[16]. Is it a bold thought, to assume that we have reached that numbers. Well, this is the main point of my post. So, I leave it there for people to reflect on.

What is Maximal Fatality Potential?

For now, my assumptions should only apply to the hardest hit countries, particularly in Southern Europe, where I believe we have already reached a Minimum Herd Immunity (MHI). This is probably best shown on the fatality rate curves. But not on the classical fatality rate (deaths/confirmed cases). Classical fatality rates, especially after this new concept of Hidden (i.e. Cellular) Immunity, are useless considering what should be the new way of counting. In my opinion, the most relevant statistics from now on will be the deaths/million (D/M) count. D/M will tell us whether a country has reached its Maximum Fatality Potential (MFP) or not. I presume that those countries that had > 500 D/M at the peak of the curve and that now are getting close to 0 D/M (e.g. Belgium, Spain, Italy, France, maybe UK) could be suitable candidates to claim the Herd Immunity status (the now famous "immunity passport"). On the contrary, countries that either have not reached their MFP or are only in the

beginning of the downslope, should be maximizing the population protection measures. Otherwise, relapses, as the one recently observed in Iran, will be seen in countries that are too prompt to relax those measures.

Conclusion

Do I think the story of this disease that has caused so much pain and uncertainty across the world in recent times, can have such an apparently almost banale end? It all depends on how we look at it. The answer is definitely not, in particular, for those countries that are still fighting with upslope or stagnant D/M curves. However, I believe that the major hurdle to solving the riddle (at least from the science side) has already been cleared by the French researchers.

Lots of unknowns and uncertainties still hover about the Covid-19 pandemic, however. Some of them I will try to write about in the coming weeks. But for now let's put a cautious closure to the perhaps more important chapter of the saga, i.e., how to get a robust herd immunity with only 5-10% seroprevalence.

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