Response to: Correspondence on “HLA-DR ‘naturally’ presented peptides: you will find what you have pulsed with” by Maggi et al

We appreciate Dr Roudier’s interest and comment on our recent article.1 2 The aim of our study was to analyse the human leukocyte antigen (HLA)-DR-associated peptidome of synovial tissue (ST) and of dendritic cells (DCs) pulsed with synovial fluid (SF) or ST, to identify epitopes that trigger T cell responses in patients with rheumatoid arthritis (RA). Dr Roudier is indeed correct when he asserts that our study is biased toward the synovium. However, we consider that ours is not a ‘conception bias’ but a deliberate effort to focus our attention on a particular tissue as a source of autoantigens, incidentally the main target of the inflammatory response in RA.

It is far from our intention to claim that only synovial antigens are relevant in driving RA autoimmune responses. Our hypothesis assumes, instead, that some RA autoantigens derive from the synovium, as has been widely shown in the literature, and our goal is to find out which synovial proteins give rise to T cell epitopes. As a matter of fact, we did find six novel T cell epitopes, including two citrullinated epitopes, but most importantly, we confirmed the suitability of this approach to uncover previously overlooked RA autoantigens, such as myeloperoxidase. Given the extensive list of peptides retrieved from HLA-DR molecules, we had to introduce a second bias in the selection of the peptides to be tested for their capacity to trigger T cell responses, giving preference to parental proteins that had been described as RA autoantigens or abundantly found in ST, including citrullinated proteins, as a proof of principle. We are now undertaking the task of screening for T cell epitopes among the comprehensive set of peptides identified in our study in the search of novel autoantigens. We acknowledge Dr Roudier’s observation on the particular processing properties of HLA-DRB1*0401 molecules,3 that will be taken in consideration in further studies.

Moreover, there are a number of technical biases in our approach, two of them pointed out by Dr Roudier. First, the depletion of immunoglobulin G (IgG) from SF prior to pulsing DCs, eliminating any antigen contained in immune complexes. Indeed, we took the decision to deplete IgG and albumin as two of the most abundant proteins in SF, in order to privilege the identification of antigens that otherwise would have been masked. This may cause some antigens contained in immune complexes to disappear from the sample, but the abundance of those proteins in the SF required their depletion from the pulsing samples. Second, we discarded skin proteins as potential contaminants derived from sample handling, which is a common practice in proteomic approaches, at the cost of not considering filaggrin in our final analyses. However, no filaggrin peptide was found in the peptidome even before discarding the abundant potential skin-derived peptides, most of which corresponded to keratin.

In our experimental design decisions were made for the sake of prioritising resources and narrowing down the object of the study. These decisions certainly imply biases. The key point is, however, to be aware of these biases and the limitations that they generate when it comes to draw conclusions from a set of results, which we believe are clearly reflected in our manuscript.

‘Where RA starts, which antigens, citrullinated or not, trigger the initial events by activating T cells?’, are relevant questions that remain unanswered (and probably will for some time). We did not aspire to solve these questions altogether in a single study. Much more modestly, we expected to provide useful information about the immunopeptidome of synovial-loaded HLA-DR molecules and the suitability of this method to identify autoantigenic T cell epitopes that may (or may not) have an impact in RA initiation or progression.

As for table 1 in our manuscript, Dr Roudier’s interpretation of the genotyping information is correct.

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