ImmunoTools *special* Award 2014

Maria Teresa Fiorillo, PhD

Dept. of Biology and Biotechnology, "C. Darwin",
Sapienza University of Rome,
via dei Sardi 70, 00185 Roma

The involvement of ADAR1-RNA editing activity in autoimmune disorders

RNA editing is a post-transcriptional process resulting in the insertion, deletion or substitution of nucleotides that generates a RNA sequence different from that encoded by the genomic DNA thereby increasing the transcriptome and proteome diversification in the cell. In higher eukaryotic cells, the most common mechanism of RNA editing is the hydrolytic deamination of adenosine (A) to inosine (I) within double-stranded RNAs (1). Subsequently, the inosine is recognized as a guanosine by the translation and splicing machineries. This covalent modification is mediated by two adenosine deaminases acting on RNA enzymes, ADAR1 and ADAR2 whose function is tightly regulated according to the tissue type and developmental stage. In human, two main size forms of ADAR1 protein are known, an interferon-inducible protein of 150-kDa found in both the nucleus and the cytoplasm and a constitutively expressed truncated 110-kDa protein, almost exclusively present in the nucleus. The ADAR2 gene transcribes a constitutively expressed 80-kDa protein. Knockout of either ADAR1 or ADAR2 gene in mice is lethal, suggesting that the physiological modification in the encoded RNAs operated by these enzymes is pivotal for a normal development. In particular, ADAR1-null mice die before birth because of widespread apoptosis and defective hematopoiesis (2).

In disease pathogenesis the involvement of RNA editing is not well understood. Several human diseases have been correlated with an altered ADAR expression and/or functioning such as amyotrophic lateral sclerosis, depression, epilepsy and cancer (1,3). As for the autoimmune diseases, only few data are available associating an imbalance of ADAR activity to the pathological condition. In patients with systemic lupus erythematosus (SLE), it was documented an up-regulation of the IFN-inducible 150-kDa ADAR1 expression in T and B lymphocytes, peripheral blood mononuclear cells (PBMC) and NK cells (4). Interestingly, the altered expression of ADAR1 correlated with an improper editing of several target RNA transcripts (4,5).

This may alter the gene regulation and ultimately play a role in the initiation and propagation of SLE pathogenesis. Very recently, mutations in ADAR1 have been shown to cause the Aicardi-Goutières syndrome, an autoimmune disorder mainly affecting the brain and the skin (6).

The goal of this project is to analyze the expression and the editing activity of ADAR1 in T lymphocytes and monocyte/macrophages in pathological conditions such as Ankylosing Spondylitis (AS), Psoriatic Arthritis (PA) and Psoriasis that represent chronic inflammatory disorders sharing a common pathogenetic background (7). To this aim, PBMC derived from patients and healthy donors will be fractionated by standard procedures to obtain purified T cells (CD3, CD4, CD8) and monocytes ($CD14^{+}$; $CD16^{-}$). These cells of adaptive and innate immunity will be suitably subjected to activating and/or differentiating stimuli in presence or not of type I interferons (α and β) or interferon γ. After treatment, the cell phenotype will be analysed by flow cytometry using the Abs indicated below. The expression of ADAR1 in patients compared with controls will be analysed by RT-PCR and western blot. Finally, we will check whether in patients there is an abnormal ADAR1 editing activity, generating iper-editing or hypo-editing of specific transcripts involved in the modulation of immune functions. In summary, the RNA editing could turn out as a new epigenetic mechanism involved in regulation of immune response and thus implicated in autoimmune disorders.

References

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FITC - conjugated anti-human CD3, CD4, CD8, CD11b, CD14, CD16, CD25, CD56, CD86, Control-IgG1,

PE - conjugated anti-human CD3, CD11b, CD11c, CD80, Control-IgG1, Control-IgG2a,

APC - conjugated anti-human CD8, CD11c, CD14, CD62L, Annexin V, Control-IgG1, Control-IgG2a,

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