Guest Editorial

János Szebeni

Complement activation-related pseudoallergy: an innate response to nanomedicines acting as pseudo-viruses

DOI 10.1515/ejnm-2015-0029

This year's first issue of the *European Journal of Nanomedicine* devoted a special section to "CARPA", i.e. "complement activation-related pseudoallergy". The third issue this year provides space for additional contributions to this special section, which completes the series with nine independent (mini)reviews and other types of papers. This commitment by *EJNM* reflects the growing recognition of the theme as a central issue in nanomedicine that may embrace a volume of research and review papers. However, the originally planned single volume became two special sections in two volumes, a sign of the theme's ability for adaptation to the changing environment, a basic feature of immunity.

As a brief reminder, CARPA is an adverse immune overreaction (hypersensitivity reaction [HSR]) against certain nanoparticles exposed to blood, mostly via i.v. infusion. Although mostly harmless, the reaction, triggered by the humoral arm of nonspecific immunity, the complement (C) system, can be fatal, making it a safety issue in the R&D of nanomedicines. Accordingly, the expansion of nanomedicine unavoidably brings with it an expansion of interest in CARPA, from all corners of this field: science, clinics, pharmaceutics and regulatory.

Before walking through the papers in these special CARPA sections, it seems worth commenting on some unclear or odd features of the phenomenon. As implied in its name, the HSR is *related* to activation of the C system and not directly linked to it. Thus, C activation may not be the sole cause of HSRs categorized as CARPA, and HSRs bearing all features of CARPA may actually not arise only as a consequence of C activation. In fact, there is no clinical evidence for a linear relationship between C activation and HSRs called CARPA, while there is plenty of evidence that C activation is NOT rate-limiting to CARPA, although it may be causally involved. The CARPA concept has been maturing over the past 20 years bringing these limits and clarifications to the surface. A potential resolution of the apparent controversy in causality may lie in a broad definition of causality, which, in the case of CARPA, emphasizes that the relationship between HSRs and C activation may be, but not necessarily causal, and even if causal, the cause-effect relationship may be indirect, and highly complex.

A more semantic than scientific ambiguity lingering around CARPA, the "nano-paradox", was also highlighted in the editorial in the January issue of *EJNM* that published the first special section on CARPA. The CARPA phenomenon is the consequence of some nanomedicines being too big, rather than tiny, making them recognizable by the C system as foreign pathogenic viruses. On the other hand, as everybody knows, the word "nano" implies very small. Thus, playing with words, CARPA is due to *nanogiganticism*, and reactogenicity can be viewed as a consequence of the "*acromegaly*" of nanomedicines. Yet another playing with words brings the Greek "pseudo" into focus. As emphasized above and in most papers in the special sections on CARPA in the present and the January issue of EJNM, the phenomenon is due to the false recognition of nanomedicines by the immune system as viruses. Thus, reactogenic nanomedicines can be perceived as artificial, or "*pseudo-viruses*", which are fought by the immune system via "*pseudoallergy*". This insight and terminology (i.e., calling nanomedicines as "pseudo-viruses") may be a powerful reminder that certain nanomedicines need to be adapted to the immune system to make them safer. Another benefit is that this terminology provides additional rationale to the word "pseudoallergy", whose only basis until now is that the phenomenon is not IgE-mediated.

Going through the particular papers published in these two special sections on CARPA, the following summaries highlight their main message, pointing out the relevance to CARPA.

The first review by Tamás Fülöp et al. (published in the January issue of EJNM), presents in detail factor H (FH), one of the best known regulators of C activation in

blood, as a protein and as a possible predictor of CARPA. Inherent or acquired reduction of FH levels make patients prone for C activation, therefore determination of FH in blood represents one of the most promising biomarkers for CARPA prediction. The paper communicates an unexpected and yet unsolved issue with FH assays, namely, that there are five FH-like proteins in the blood which do not share the C inhibitory function of FH but are immunochemically indistinguishable from FH. The laboratory measurement of CARPA-predictive FH thus needs to be solved before the use of this biomarker can be assessed.

The second minireview by László Dézsi et al. focuses on CARPA in rodent models: rats, mice, guinea pigs and rabbits. Information on all aspects of hypersensitivity reactions caused by known complement activators (zymosan, cobra venom factor) and different nanomedicines (liposomes, other drug carrier nanocarriers) in these species has been compiled and analyzed, in an effort to highlight the similarities and differences. The review also compares the murine model with the pig model and human data, detailing the hemodynamic, cardiopulmonary, hematological and blood chemical changes. One of the most important messages is that rats are 2–3 orders of magnitude less sensitive to liposome-induced CARPA than pigs or hypersensitive humans. Thus, CARPA can be studied in rodent models, but they do not necessarily mimic human CARPA.

The third minireview by Domokos Csukás et al. focuses on pulmonary intravascular macrophages (PIM cells) which are prime candidates to be the cellular mediators of porcine CARPA. As detailed in the review by Urbanics et al. (see below), pigs provide the most sensitive animal model for CARPA, whose sensitivity equals that of humans who are hypersensitive to a reactogenic drug, such as Doxil® or liposomes. PIM cells, which are abundantly present in the lungs of pigs and represent a macrophage subpopulation, do seem to explain this high sensitivity of pigs to CARPA. The review details all known unique qualities of PIM cells giving rationale to find similar cells in hypersensitive humans.

The fourth review by Gergely Milosevits et al., the first review in the present volume, gives a comprehensive description of exosomes, natural liposomes released by cells, which are fashionable research objects in the frontier of molecular-cellular biology today. Exosomes play important roles in intercellular messaging and other functions in health and disease, which are detailed in the review, along with their potential utilization as a stealth drug delivery system. Exosomes have particular relevance in CARPA because they look and act like liposomes, they become exposed to blood, yet do not activate C. Hence, nature equipped them with an efficient defense system against C, whose understanding may be highly educational for nanomedicine developers trying to formulate C-stealth nanomedicines.

The fifth review by Rudolf Urbanics et al. gives a detailed description of the porcine model of CARPA, illustrating for the first time the constant and variable endpoints of the assay. As mentioned above, pigs provide the most sensitive animal model for CARPA with sensitivity equaling that of hypersensitive humans, and with symptoms identical, or very similar to those displayed by man undergoing CARPA. Therefore the pig model continues to capture the interest of all who work on clinically applicable liposomes or other nanomedicines. In fact, based on the growing regulatory requirements for preclinical safety assays for CARPA, many features of the porcine model make it the best candidate to become the industrial standard for large animal drug screening and prediction of CARPAgenic potential of (nano)medicines. The review provides unprecedented details of the setup and techniques for measuring the different CARPA endpoints. In addition, it outlines other uses of pigs as disease models.

The sixth minireview by Zsófia Patkó and János Szebeni deals with what is a standard part of all in vivo research papers on CARPA but is rarely addressed in terms of mechanism: the blood cells changes during CARPA. Leukopenia with reactive leukocytosis, thrombocytopenia, occasional lymphopenia are present in variable degree in all animal models of CARPA, and are explainable with various anaphylatoxin effects on these cells. These blood cell changes reflect complex interactions among leukocytes, platelets and endothelial cells, which are a major contributor to the pulmonary hypertension and systemic blood pressure changes in CARPA. The formation of monocyte-platelet aggregates, leukothrombi in the pulmonary microcirculation represent major and mostly unknown processes CARPA. The review discusses the major cell surface receptors and ligands playing a role in these processes. By enlisting some inhibitors of these interactions the review presents ideas on how to prevent these cell changes in CARPA.

The seventh paper, a short communication by Yorulmaz Saziye et al. presents a state-of-art approach of real-time measurement of C activation in a potential bedside assay of CARPA. The nanodevice is based on molecular quantitation of the build-up of the membrane attack complex of C (C5b-9) on supported bilayer membranes, the final step of C activation. As emphasized previously, C activation may be causally involved in CARPA in a complex manner, and there is mounting evidence that detection of rapid and strong C activation in the blood of patients, or in screening

serum of patients incubated with the reactogenic nanomedicine, is predictive of CARPA. Successful engineering of a CARPA predictive bedside assay is likely to become a major milestone in preventing the problem, and the preliminary data published in this paper will hopefully expedite efforts to bring the approach to success.

The eighth paper, a short communication by Tamás Mészáros et al. presents an unprecedented and unexpected observation on a paradoxical rise of hemolytic C in the blood of mice soon after i.v. injection of zymosan- and liposomes. The significance of the observation lies in the question, whether it represents a reproducible and explainable endpoint of CARPA that would lend credence to the mouse model for CARPA tests and screening. The pilot study gives affirmative answers to both these questions, providing rationale for further experiments to obtain direct evidence in support of our present hypothesis explaining the phenomenon. According to this hypothesis, anaphylatoxin-induced hemoconcentration, due to capillary leakage, leads to the transient relative rise of C3 in the blood of treated mice, which overwhelms C depletion. The study also reports extended sensitivity of apolipoprotein E-deficient (ApoE KO) mice for C activation-related paradoxical C3 rise, suggesting the use of these animals as a mouse model.

The final commentary in this special CARPA section by Moein Moghimi et al. brings us to the mechanistic aspects of C activation during CARPA. The authors point out that the array of surface extensions on certain functionalized drug carriers provide these nanoparticles 'pathogen-mimicking' properties that trigger C activation via the lectin pathway. Examples include PEGylated liposomes, nanospheres, micelles and carbon nanotubes, polysaccharide-coated superparamagnetic iron oxide nanoparticles, poloxamer and poloxamine block copolymers. The concept represents a new paradigm in the C field, as lectin pathway C activation by nanoparticles has not been known in the literature before. The observations are not only exciting scientifically, but they open new possibilities to prevent CARPA, by inhibiting lectin pathway activation of C. Also, evaluation of lectin pathway components in the blood of patient may serve as a CARPA biomarkers predicting reactions.

Taken together, the 9 papers introduced above illustrate the richness, and at the same time, limitlessness of scientific and clinical questions, issues, unsolved problems in the field of CARPA. It is hoped that they capture the interest and imagination of the readers.

János Szebeni, Nanomedicine Research and Education Center, Semmelweis University, Budapest 1089 Nagyvárad tér 4, Hungary; and Faculty of Health, Department of Nanobiotechnology and Regenerative Medicine, Miskolc University, Miskolc, Hungary, E-mail: jszebeni2@gmail.com