Dendrimeric-Nanocarriers for easy *in vitro* detection of allergic reactions induced by β-lactams

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Immediate allergic reactions to β–lactams are mainly diagnosed by a compatible clinical history and the presence of skin test positive and/or *in vitro* test to different β–lactams determinants. In some cases, when skin tests are negative, a controlled administration of the drugs may be necessary although neither is free of risk, especially in persons with anaphylactic reactions. The determination of specific immunoglobulin E antibodies, IgE (antibody associated with an allergic response) in serum is a very valuable method to confirm the diagnosis, although it is not as sensitive as skin testing. An improvement in the *in vitro* techniques is therefore needed to enhance diagnosis in allergic patients. Diagnostic testing in the immunological setting is primarily concerned with the recognition and confirmation of the presence of IgE antibodies.[1] The analytical challenges of an *in vitro* test are therefore related with sensitivity (i.e. lowest detectable concentration) and specificity (i.e. accuracy for the analyte or group of analytes required). The analytical sensitivity and specificity have an important influence on the clinical sensitivity and specificity of the method. The binding of an immunoreactive component such as an analyte-specific antigen to a specific antibody immobilized on a solid-phase support is the essential, common feature of solid-phase immunoassay techniques. Such techniques are used to measure circulating levels of a number of markers used as guidance in the management of patients with specific clinical symptoms. For example, the RadioAllergoSorbent Test (RAST) shown in Figure 1 is a blood test used to determine what a person is allergic to, based on the amount of IgE reacting specifically with suspected or known allergens.[2]

Conjugation of BL to a macromolecular carrier has been used as a tool for the *in vivo* and *in vitro* tests for diagnosing IgE mediated reactions. Classical conjugation of HSA (Human Serum Albumin) with penicillins generates the benzylpenicilloyl determinant (BPO), the major antigenic structures.[3]
PLL (Poly-L-Lysine) is a versatile homopolymer that has been extensively used as a precursor of drug-polymer conjugates.[4]. However this approach, due to differences in the degree of polymerization and subsequent functionalisation frequently lacks of reproducibility.

Dendrimers can be considered the most versatile, compositionally and structurally controlled synthetic nanoscale building blocks available today.[5]. Artificial antigens have been synthesized and they have shown that these hapten dendrimer conjugates are recognized by IgE directed to BL, emulating the *in vivo* drug conjugates to proteins.[6,7]

The shift from cellulose (fibrous and rough surface, with holes that can host the dendrimer) to nano/microstructure transparent surface as zeolites seems to be an important improvement in our system.

Zeolite L is a microporous crystalline material featuring a one dimensional channel system. The length of these cylindrically shaped crystals can be tuned from 30 nm to about 10 microns. Zeolite L can be prepared with different aspect ratios (i.e. length / diameter),

forming either thin discs or long cylinders. [1] A. Zabala Ruiz, D. Brühwiler, L.-Q. Dieu, G. Calzaferri, in Materials Syntheses, A practical Guide, Eds. Schubert U., Hüsing N., Laine R., Springer, Wien, (ISBN 978-3-211-75124-4), 2008, 1.

The surface of zeolites L has been functionalized with hapten dendrimer conjugates. The novel organic-inorganic hybrid materials have been characterized by IR, XPS, zeta potential and ninhydrin test proving the successful covalent anchoring of the dendritic macromolecules to zeolites L. RAST analysis using them is going to be done as soon as possible.

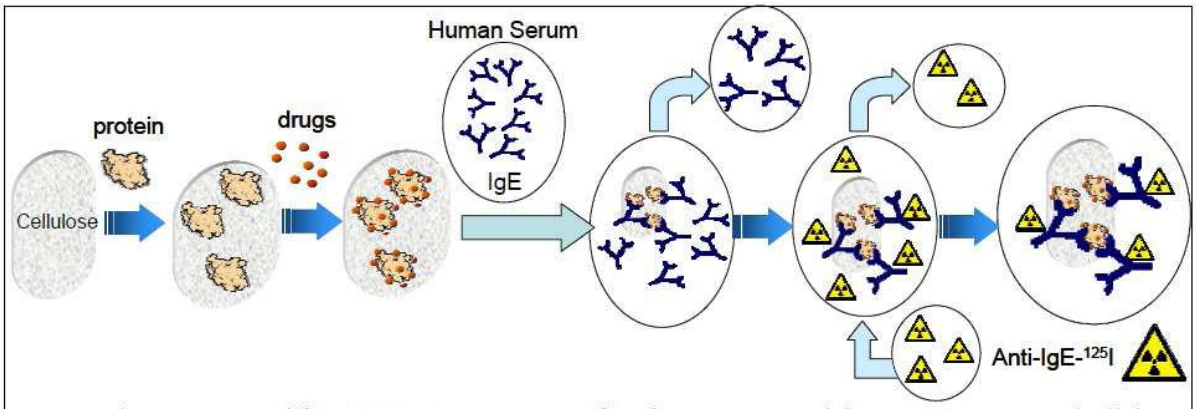

Fig. 1. The main steps of the RAST test are summarized in the cartoon. A Solid Support consisting of cellulose discs, is modified with a protein and the suspected allergen. After treatment with the human serum, the IgE attached to the allergen (responsible for the allergic reaction) is bonded to anti-IgE labelled with ^{125}I. The quantification of the attached IgE is made by measuring the disc radioactivity.

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