

## **IP Internship project 2:**

### **Characterization of IgG dimers/polymers in therapeutic immune globulin preparations**

**Introduction:** Patients who are immunoglobulin deficient receive intravenous immunoglobulin (IVIG). People with autoimmune diseases and viral infections also benefit from IVIG therapy. IVIG contains mainly monomeric immunoglobulin G (IgG) but also contains a few percent IgG dimer and IgG polymer. The effects of the presence of IgG dimer are not entirely clear: in certain conditions it can lead to a better efficacy of the administered IVIG, while in other diseases the effect is rather negative. Moreover, the structure of the dimers (head - head or tail - tail) is still unknown. The presence of polymeric IgG in IVIG could lead to anaphylactic shock in some patients, which is why during IgG purification protease is added to prevent polymerization. For the IgG polymers it is also not exactly known how they are formed. The manufacture of and research on IVIG (both in powder form and the new liquid product) is a point of attention of Sanquin for the coming year.

Immunoglobulins consist of two functional parts: two identical fragments both of which can bind antigen (Fab) and a crystalline (Fc) part that interacts with receptors and is involved in signal transduction. Rheumatoid factor (RF) is defined as an IgM (occasionally IgG or IgA) antibody targeted at the Fc fragment of IgG, in other words, it is an auto-antibody. These IgM-IgG immune complexes are mainly found in the joints of rheumatoid arthritis patients. RF preferentially reacts with polymer IgG

**Aim:** To investigate how RF binds to IgG dimer and IgG polymer. First you will try to make IVIG with extra dimers/polymers, also known as IVIG in immune-complexes, in different ways (change in pH, addition of salt, etc.). This will be examined by RF linked to Sepharose. It will be measured if IVIG-labeled complexes (and, as a positive control, anti-tetanus IVIG complexes with <sup>125</sup>I labeled tetanus) bind to the RF Sepharose. Alternatively, a mixture of allergen and serum of allergy patients can be added to RF Sepharose after immunotherapy, since such allergy patients' serum contains both IgG and IgE which can form an immune complex on addition of allergen. This immune complex contains both IgG and IgE, where binding of the IgG component in the complex of RF-Sepharose is detected with <sup>125</sup>I-labeled anti-IgE (as in a RAST).

As a side project, the ratio IgG4/IgG1 is determined in many sera of allergy patients who have had immunotherapy.

#### **Techniques**

- RAST (radio allegro sorbent test)
- Working with radioactive labeled materials
- Working with patients' serum
- Separating and characterizing proteins with SDS-PAGE
- Also possible: separating of IgG fractions with FPLC with different chromatographic principles; detection of binding between molecules with Biacore.

**Duration:** to be discussed. Students from the University or HLO who are looking for a dynamic and interesting internship and are interested in the above project are encouraged to contact the group leader, Theo Rispens, either by e-mail: [t.rispens@sanquin.nl](mailto:t.rispens@sanquin.nl).