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trans2care
Transregional Network for Innovation and Technology Transfer to Improve Health Care

Early diagnosis of coeliac disease

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Abstract — At the Immunopathology Laboratory at the IRCCS Burlo Garofolo Hospital the research activity is based on autoimmune diseases, above all on celiac disease in order to diagnose it in an early stage. For this reason, we are collecting many serum samples and intestinal biopsies to analyse them with molecular (phage-display) and immunofluorescent (double staining and activated beads) assays. Within the Trans2Care project we intend to apply these methods in several areas related to the problems explored by the Project partners with the aim of promote collaboration, mobility of researchers and exchange of knowledge between partners.

Index Terms — autoimmune diseases, immunofluorescent assays, intestinal biopsies, phage-display analysis, serum samples

1 THE IRCCS BURLO GAROFOLO

The Burlo Garofolo Hospital was established on November 18th 1856, when the Spedale Infantile was instituted in order to grant free medical care to poor children. On 1968 the Institute was designated by the Ministry of Health as IRCCS. The Institute has promoted and implemented an innovative health culture, based on innovative policies aimed at reduction of hospital stay and humanization of medical care. The Hospital is the unique health care Institute for Mother and Child Health within the area the sourrounding region. The IRCCS Burlo Garofolo ensures clinical excellence in medical and surgical paediatric subspecialties, reproductive medicine and perinatology. The Institute offers graduate and post graduate courses and PhD programs. The Institute is a World Heath Organisation (WHO) Collaborating Centre for Maternal and Child Health. Fundamental, clinical, epidemiological and health services research are organised along 6 main subjects: Maternal and foetal medicine; neonatology; chronic diseases, including cancer, with onset in paediatric age; paediatric surgical and rehabilitation sciences; epidemiology prevention and quality of care; neuroscience in developmental age.

2 IMMUNOPATHOLOGY LABORATORY

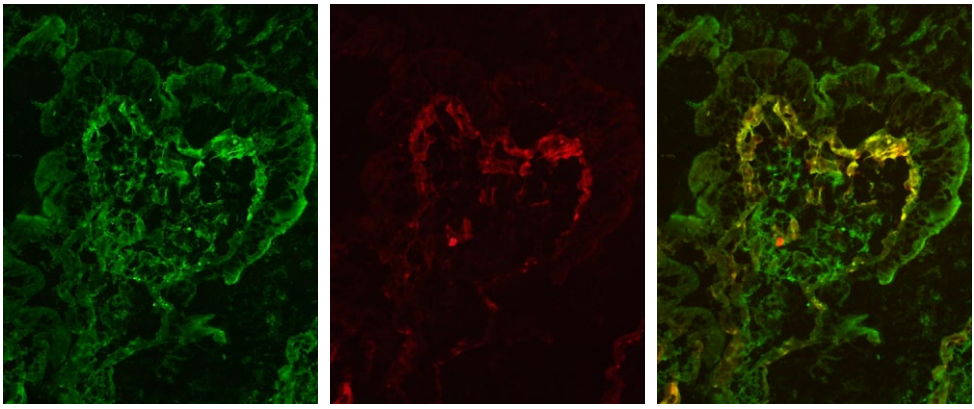
The Immunopathology Laboratory has one professor and one assistant professor in clinical pediatrics, four PhD researchers. We use and develop immunohistochemical and molecular techniques to study autoimmune diseases to understand its inflammatory cascades. Furthermore, we apply at the patient's bed side our assays to simplify the diagnosis of these pathologies. The research work is financed through national research programme schemes (NHS programme 2009: "Dilatative cardiomyopathy and gluten dependent autoimmunity"; Italian Ministry of University 2009: Anty-idiotypic network to anti-transglutaminase antibodies in the pathogenesis of celiac disease; XVII Executive programme of scientific and technology co-operation between Hungary and Italy 2010: LS17 Intestinal gluten-dependent immune response in the early stages of celiac disease) and from 2010 we are partners in an international strategic project Trans2Care.

2.1 Research Activities

Our interests are based on autoimmune disorders [1-3]. We organised a serum and tissue bio-bank from patients suffering from organ specific autoimmune disorders (e.g. type 1 diabetes, thyroiditis, celiac disease, rheumatoid arthritis) or from other inflammatory diseases (e.g. Crhon disease, eosinophyl-gastritis, ulcerative colitis) with a large samples stored at -80 C° (10000 serum samples and 1500 intestinal biopsies).

Our main goal is to study celiac disease (CD) [4-8], an autoimmune-mediated enteropathy characterised by gluten-triggered small bowel mucosal lesions in genetically susceptible individuals carrying the CD-related human leukocyte antigen (HLA) DQ2 or DQ8 haplotypes. The current diagnostic criteria for CD require intestinal mucosal villous atrophy and the presence of serum antitransglutaminase (anti-TG2) antibodies [9-13], even if many patients suffer from gluten-dependent gastrointestinal symptoms before the onset of villous atrophy and of anti-TG2 antibodies in serum. Anti-TG2 antibodies are synthesised by specific B lymphocytes in the small bowel mucosa and they are deposited in the morphologically normal small intestinal mucosa before they can be detected in the circulation. Starting from these evidences, we try to identify these antibodies working on intestinal specimens. Creating phage-antibody libraries against TG2, we observed that celiac-specific anti-TG2 antibodies are primarily comprised of the IGHV5-51 gene from the VH5 antibody variable gene family, indicating a possible preferential usage of this gene in the gluten-dependent autoimmune response to TG2. Indeed, using phage-antibody libraries against TG2, we demonstrated that a large proportion of HLA DQ2- or DQ8-positive relatives of CD patients produce anti-TG2 antibodies in the intestine as a response to gluten, even in the presence of normal intestinal morphology and when no anti-TG2 antibodies can ever be found in the serum [14].

We are also able to investigate IgA anti-TG2 in frozen biopsies by using two other assays easier and faster than phage assay: the double immunofluorescence staining (Fig.1) and the IgA anti-TG2 antibodies quantification through TG2 activated beads and flow cytometric analysis.



A B C

Fig.1. Criosections from a celiac intestinal biopsy: (A) green signal for IgA, (B) red signal for TG2, (C) yellow signal for IgA deposits co-localised with TG2.

3 ROLES IN TRANS2CARE

3.1 What offer to T2C

We have focused our research project in the diagnosis of celiac disease in an early stage using immunofluorescent assays and phage-display analysis. We provide our knowledge to T2C partners, in order to facilitate the exchange of expertise and to study tissue biopsies may also be different from intestinal ones.
As described in the paragraph above, we have an elevated number of biological samples (sera and intestinal specimens) suitable for exploration of large data sets.

4 CONCLUSION

Through Trans2Care project, we intend to contribute with our expertise in order to reach the common goal, that is to establish collaborations and connect the project partners to eventually improve their expertise and to promote exchange of ideas and technology transfer.

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