CytoGenex *at the Well-One Clinic.....*

June 20th 2011 Issue 1

CytoGenex -The Well-One Clinic

Although work continues to address quality and safety parameters for the food and nutritional supplements industry, CytoGenex is developing a program of Clinical Research and testing for chronic disease. In association with Dr Beryl Beynon of the Well-One Clinic, Beverley, East Yorkshire, work began in November 2010 to address the issue of Lyme disease. This research work is part funded by The Jacob's Well charity Beverley and samples are provided by Dr Jean Monro, Medical Director of the Breakspear Hospital, Hemel Hempstead, Hertfordshire.

Lyme Disease-new Mikrogen Assay

Currently the diagnosis of Lyme disease is made by clinical symptoms. Laboratory conformation is based on serology and the detection of IgM and IgG antibodies to various antigens of the spirochete, Borrelia *burgdorferi sensu lato*. CytoGenex is currently undertaking an evaluation of a new assay format from the German company, Mikrogen. It is hoped that the work will be completed by the end of the summer. For more information on Lyme disease see: http://www.lymediseaseaction.org.uk/

Director of Clinical Research Appointed

Dr Cyrus Karimi has recently been appointed to take-over the running of the new Clinical Research program at CytoGenex. Dr Karimi is a medical doctor with 15 years of clinical experience. In addition to his clinical work, Dr Karimi has a strong familiarity with laboratory procedures.

CytoGenex- New Immunodiagnostic Research Laboratory opens in Hull, North Humberside

Contents and format for future issues:
This first issue of the CytoGenex newsletter focuses on the MELISA® methodology for detecting immuonsensitivity to environmental agents. As a format, future issues of the newsletter will outline ongoing activities for the laboratory and issues of interest to the laboratory staff, both from archive (published/unpublished) research studies, ongoing projects and proposed projects.

MELISA® (Memory Lymphocyte Immuno-Stimulation Assay)

In association with the MELISA® Medica Foundation, CytoGenex is now able to provide for the first time in the UK, the premier diagnostic test for identifying hypersensitivity to metals and other foreign materials (food allergens/microbial pathogens). The immune changes induced by hypersensitivity to metals, allergens and pathogens disturbs the neuro-endocrine-immune system (NEIS©) and may be the route cause of both acute and chronic ill health.

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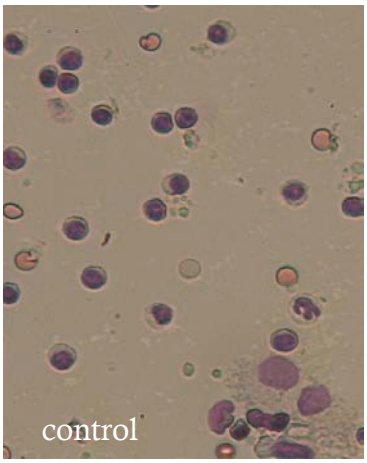
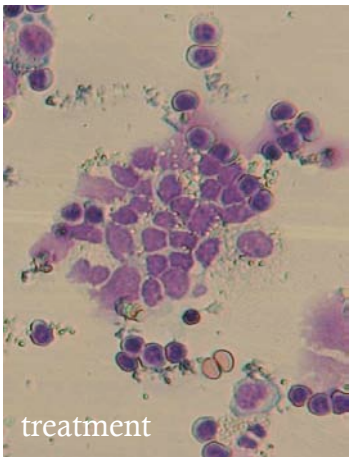
MELISA® can be used to detect allergy to a range of substances/agents

- Cosmetics and jewelry
- Chronic infectious disease (Lyme)
- Dental amalgams and implants (metals)
- Food allergens (e.g. Gluten) and cookware (metals)
- Occupational exposure (heavy metals)
- Orthopaedic and electronic implants (metals)
- Preservatives and fillers in medications and vaccinations (organic metals/metal oxides)





MELISA® procedure

1. Blood drawn from subject and transported to laboratory (24h special delivery)
2. Memory T-lymphocytes concentrated and exposed to allergens
3. Cells incubated for 5 days at 37°C
4. Number of T-lymphocytes is measured by direct staining (microscopy) and by a quantitative procedure
5. Results reported to patient/clinic as stimulation index (SI). SI >3 is a positive response.

MELISA® continued from cover page

It is now recognized that inflammation plays a role in disease processes as widely diverse as minor cuts and bruises, to cancer (1). An inflammatory condition is very often provoked by environmental factors such as pathogenic microorganisms and food components/foreign substances (allergens). Inherent in the process is the activation of the immune system. The immune system is composed of two fundamental elements; the production of immunoglobulin antibodies by B-lymphocytes and the activity of cells such as macrophages and natural killer cells (cell-mediated immunity). When an invading pathogen is detected, the cell-mediated immune system is at hand and this can be reinforced by the production of antibodies from B-lymphocytes. For some individuals and by a mechanism that is not entirely understood, prior exposure to a pathogen or foreign substance can lead to a hypersensitivity

MELISA® is designed to detect type IV hypersensitivity (allergic reactions)

reaction, when the individual is next exposed to the agent. This type of sensitivity, called type IV hypersensitivity, involves T-lymphocytes that have a memory of previous exposure to the agent. Type IV hypersensitivity can lead to an allergic reaction to metals, food components, fungal and bacterial agents. If it is suspected that an individual is sensitive to an environmental factor, the MELISA® is designed to confirm this. The MELISA® belongs to a class of assay system called the Lymphocyte Transformation Test (LTT), however, most assays of this type suffer from a lack of sensitivity as the number of memory T-lymphocytes in the blood is rather low. The MELISA® has solved this problem by increasing the proportion of memory T cells by a special centrifugation process. The result of this procedure is an enriched fraction of memory T-lymphocytes (2). These cells can then be incubated with the material that is thought to induce an allergic reaction. After a period of 5 days, cells are labeled to determine whether there has been an increase in number of the T-lymphocytes. Studying the treated and control cells under the microscope confirms the effect: a typical response is displayed in the panel to the left above. The increase in the number of T-cells is recorded as a stimulation index (SI). Positive responses have an SI of 3.0, where the number if T-lymphocytes is three times the control.

MELISA® continued from page 2

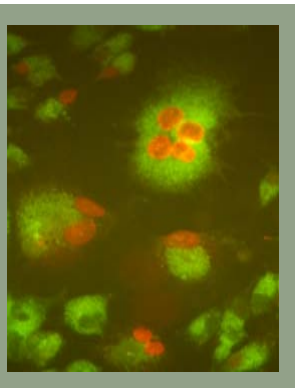
In addition to its use in determining sensitivity to allergens, the MELISA methodology might have a significant role to play as a laboratory diagnostic test for infectious agents such as Borrelia *burgdorferi*, the bacterium responsible for Lyme disease. Studies conducted on samples from patients with suspected Lyme disease using the new Mikrogen lineBlot serology assay, are indicating that a large proportion of patients are negative for antibodies to Borrelia. Of particular significance is the finding that where serology is negative, the MELISA can give a positive result. These preliminary data suggest the need for a wider comparison of the Mikrogen serology assay with the T-lymphocyte stimulation assay using a large population of patients with suspected Lyme disease. The results of this study could have a strong influence on the rationale for the current two-tier serology protocol that was originally proposed in the mid-1990s (3).

Abstract of CytoGenex Research in Collaboration with the Max-Planck Institute Munich on Bacteria Activating Immune Receptors on Cancer Cells

Blood-borne bacteria, fungi and viral agents can activate cells of the innate immune system by interacting with pattern-recognition or Toll receptors on the surface of immune cells. We demonstrate here that mRNA for Toll receptors is ubiquitously expressed in a range of transformed and normal cell types. These findings raise the possibility that infection could induce an inflammatory response in somatic tissues and this might 1), provide a milieu for changes in normal cells that lead to neoplastic growth and/or 2), that it might provide conditions suitable for enhancing the growth of an existing neoplastic lesion. As a preliminary step to investigate this, we have studied the response of breast tumour MCF-7 cells to a sonicate of a mixed bacteria cell population. Using quantitative PCR (QPCR) and primers for Toll receptors 1 to 10, we have shown that 24 h exposure to the bacterial cell sonicate up-regulates the expression of mRNA for Toll-2 and Toll-4 by between 5 and 8 fold. The expression of other Toll receptors was not significantly altered. Given that the cytokine IL-6 is induced in immune cells by ligands of Toll receptor 4, we have further demonstrated by QPCR, that mRNA for this inflammatory cytokine is markedly induced in MCF-7 cells by exposure to the sonicated bacteria and that this effect is blocked by prior exposure of the MCF-7 cells to dexamethasone. These finding strongly suggest that the full Toll-mediated inflammatory system is present in breast tumour cells and they provide a rationale for tumour therapy, targeted to the Toll receptors.

Funding of Research

All basic research laboratory studies are funded partly by fee-for-service work generated by CytoGenex and partly by Jacob's Well Medical Research. Donations to the charity would be most gratefully received. For more information see The Jacob's Well website at: http://www.thejacobswell.org/index.php



News and publications .....

Methodology/systems development

- Methodology is being established for the growth of the feline calca virus as a surrogate for Norovirus and the development of antiviral agents
- Work continues on the testing of the Mikrogen lineBlot assay for the detection of Lyme Borrelia
- An HPLC methodology for the detection of plasma and tissue levels of ascorbic acid (Vitamin C) is being developed
- A novel treatment system is being explored for testing against antibiotic resistant strains of bacteria
- Abstract submitted and accepted by Society of Endocrinology: Toll receptor-mediated inflammatory system is present in tumour cells from endocrine-related tissues (see abstract in the left panel)

Events.....

- James Lind Alliance meeting on Lyme disease held at the Academy of Medical Sciences, Portland Place London, attended by Drs Beynon and Newton; full report in future issue
- ILADS education meeting in Augsburg Germany 28-29th May attended by Dr Chris Newton from CytoGenex; full report in future issue
- Dr Newton visited the Max-Planck Institute for Psychiatry Munich, 30th -31st May

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Metals Activate Toll Receptors

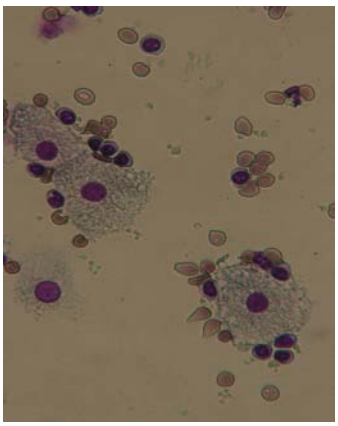
A skin response to Nickel (Ni) is one of the most common causes of contact allergies. Studies have demonstrated that Nickel and Cobalt can activate pro-inflammatory pathways in cells, however, the mechanism by which they do this has not be known (4). Now studies presented in the journal, Nature Immunology, suggest that Ni can bind to special proteins on the surface of what are termed antigen-presenting cells. These protein receptors belong to a class called pattern recognition receptors, the best known of which are the Toll-like receptors (TLR). These receptors are thought to provide a first line of defense again marauding bacterial pathogens such as E. coli, as they can signal to T cells that a pathogen is present. The work by Marc Schmidt and his colleagues in Mannheim, Germany has shown that Ni can directly activate TLR-4. This is the protein receptor that is activated by bacteria like E. coli. The effects that this group observed were species specific; Ni did not activate the mouse TLR-4. Detailed studies where the TLR-4 receptor protein was modified showed that two amino acids of the human TLR-4 protein were essential for activation by Ni. These two histidine amino acid molecules were not necessary to signal the presence of bacteria such as E. Coli, as the modified TLR-4 still responded to lipopolysaccharide, a molecule in the cell wall of gram-negative bacteria such as E. coli. The authors raise the possibility of being able to specifically interfere with this Ni-binding site to prevent an allergic response without altering the vital role that TLR-4 plays in responding to pathogens. In a future issue of this newsletter, we will address the involvement of metals in chronic disease such as, Multiple Sclerosis, Alzheimer's disease and Cancer.

Metal oxides; from toothpaste to toast

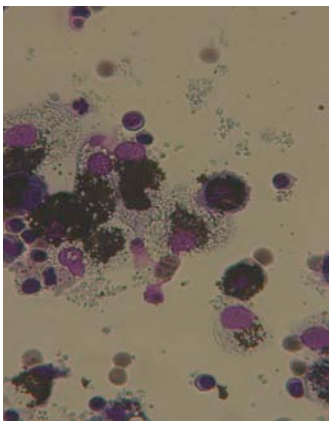
Next time you scrub your teeth, take a look at your toothpaste packet and you might be a little astonished to find that the paint-whitening agent, Titanium dioxide (TiO_2) is one of the ingredients. So perhaps you would not be surprised to find that TiO_2 is often

TiO_2 continued.....

put into flour, milk powders and cheeses. This compound is a food additive as indicated in GSFA Table 3 Provisions of the CODEX *alimentarius* WHO food standards (6). Our studies in the laboratory show that TiO_2 is extremely insoluble, in other words it does not readily dissolve and stays as fine particles of about 0.1-0.5 μm in diameter. When the antigen presenting cells, the macrophages, come into contact with TiO_2 the picture below shows what happens- they engulf the insoluble particles (see black areas within macrophages in comparison to grey granulated appearance of untreated, control macrophages). Current lab. studies are addressed to events subsequent to uptake of the particles. Recent evidence has been presented by Andrew Islam and colleagues, at Case Western Reserve University, that Titanium particles activate TLR-4 receptors (7). It remains to be observed as to whether the oxide form can do the same.



Control untreated cells



Cells treated with TiO_2

References used in this issue 1. Couussens and Werb. Natue Dec 19-26;420(6917):860-7 (2002). 2. Valentine-Thon et al. Neuron Endocrinol Lett 1: 17-24 (2006) 3. Thomas et al. Journal of Clinical Microbiol. 34: 2343–2350 (1996) 4. Goebeler at al. J. Immunol 155, 2459-2467 (1995) 5. Schmidt et al. Nature Immunology August 15, 1-7 (2010) 6.http://www.codexalimentarius.net/gsfaonline/additives/details.html?id=184 7. Islam et al. J Orthopaedic Reseach 29. 211-217 (2011)

If you would like any more information on issues raised in this newsletter or you would like to make a contribution to a future issues, please do not hesitate to contact CytoGenex or the Well-One Clinic at the address given below

Postal addresses/telephone number for contact:

CytoGenex at the Well-One Clinic
The Jacob's Well Yard
Swinemoor Lane
Beverley
Easy Yorkshire
HU17 0BX Tel: 01482 870490

Email addresses

cjnewton @cytogenex.com
office@thejacobswell.org