

Introduction

The Molecular Immunology unit is developing and evaluating new technologies for the design and immunological testing of candidate vaccines, analysis of the genetic and antigenic diversity of pathogens and the contribution of the immune system to the efficacy of therapeutic interventions. This research focuses primarily on three infectious diseases: (i) malaria due to *Plasmodium falciparum*, (ii) bacterial meningitis caused by *Neisseria meningitidis* and *Streptococcus pneumoniae*, and (iii) *Mycobacterium ulcerans* disease (Buruli ulcer).



Picture of the Molecular Immunology Group. (Photo M. Tamborini)

3.1 *P. falciparum* malaria: developing a synthetic subunit candidate vaccine

The development of a malaria vaccine currently represents one of the most important scientific challenges in global public health. One approach is to design a subunit vaccine that incorporates several malaria protein antigens for which there is evidence of protective immunity from epidemiological data or experimental animal challenge models. At present, the development of such subunit vaccines is hampered by a lack of antigen delivery systems to drive suitable protein antigen-specific immune responses in humans that are both highly effective and human-compatible and have an appropriate safety profile. Production of synthetic or recombinant proteins that stably mimic the native structure of the corresponding malaria antigens to induce effective humoral immune responses is a further major challenge. Since no vaccine against a human parasitic disease is currently available, novel approaches and technologies are required to develop such vaccines.

Clinical testing

In cooperation with Pevion Biotech (R. Zurbriggen) and the Institute for Organic Chemistry, University of Zurich (J. Robinson), we are developing a candidate malaria vaccine based on synthetic peptides displayed on the surface of immuno-potentiating reconstituted influenza virosomes (IRIVs). IRIVs represent an innovative antigen delivery system derived from a mixture of natural and synthetic phospholipids and influenza surface glycopro-

teins. We have demonstrated that IRIVs are suitable for eliciting strong immune responses against peptide antigens attached to their surface via phospholipid anchors. Using an iterative antigen optimisation process, we have created synthetic peptides that mimic the native structure of surface loops of two key *P. falciparum* vaccine candidate antigens, the circum sporozoite surface protein (CSP) of sporozoites and the apical membrane antigen 1 (AMA-1) of merozoites. In a phase I clinical trial we have demonstrated the safety and immunogenicity of the two components, designated PEV301 (AMA-1) and PEV302 (CSP), given in two different doses alone or in combination. At appropriate antigen doses, following two injections both vaccine components elicited long-lived peptide-specific immunoglobulin G (IgG) antibody responses in all volunteers immunised. IRIVs seem to have both an immunopotentiating adjuvant-like activity and to act as a carrier system that provides T cell help to malaria antigen-specific B lymphocytes via influenza antigen-specific T cells. Correlations between high-parasite-binding IgG titres with positivity in PEV301 peptide-specific lympho-proliferation assays indicate that malaria peptide-specific T cells can provide additional T cell help. Importantly, all volunteers showed pre-existing influenza antigen-specific immune responses that did not negatively affect the vaccine-induced humoral and cellular immune responses. Purified immunoglobulins from volunteers immunised with PEV302 inhibited sporozoite migration and invasion of hepatocytes in vitro. Combined delivery of the two IRIV-formulated peptides did not interfere with immunogenicity of either peptide, demonstrating the suitability of the IRIV system for developing multivalent (that is, comprising several antigens) subunit vaccines.

In a next step PEV301 and PEV302 were tested in a phase IIa clinical trial with the main outcome measure being protection from malaria in a sporozoite challenge model. We observed evidence of blood-stage immunity in PEV301/302-vaccinated volunteers, but no complete protection from malaria. Although there was no sterile protection for the volunteers, two major observations indicate an effect of the vaccine-induced response on blood-stage parasites: (i) Lower rates of parasite growth were observed in vaccinated volunteers compared with unvaccinated controls, and this was reflected in parasite-specific polymerase chain reaction (PCR) results. These showed early control of parasitaemia by some volunteers in the PEV301/302-vaccinated group. (ii) Morphologically abnormal parasites were present in the blood of all vaccinated volunteers and in significantly fewer controls ($p=0.001$). These findings constitute the first evidence of vaccine-induced blood-stage efficacy in a sporozoite challenge study and were the basis for conducting a subsequent clinical trial in the main target population, children living in African countries where malaria is endemic. This phase Ib trial began in January 2008 at the Bagamoyo Research and Training Centre (BRTC) in Bagamoyo, Tanzania (see section 12, SCIH, Pharmaceutical Medicine unit).

Developing additional components for a multivalent malaria vaccine

Since we assume that an effective malaria subunit vaccine has to incorporate more than two antigens, we are developing peptidomimetics of additional key *P. falciparum* vaccine candidate antigens. Pre-clinical profiling of new components targeting the antigens merozoite surface protein 1 (MSP-1), MSP-3, SERA-5 and the newly identified candidate antigen D13 is currently being finalised.

The search for new vaccine candidate antigens is focused on predicted glycosyl-phosphatidyl-inositol (GPI)-anchored proteins and proteins involved in an interaction network with MSP-1 and D13. Based on a set of criteria, such as expression patterns, predicted protein-protein interactions and the presence of protein domains of particular interest, we have selected sets of predicted *P. falciparum* proteins for heterologous expression and the production of specific serological reagents. These are used to study expression patterns at the protein level, protein processing, subcellular localisation and biological functions. Moreover, employing transfection and homologous recombination in *P. falciparum* asexual blood stages, we will investigate the biological function of D13 in parasite growth regulation. Finally, to complement the genetic approaches and immunofluorescence analyses, we have also devised quantitative proteomics techniques based on stable-isotope labelling with amino acids in cell culture followed by mass spectrometry.

The overall goal of our malaria vaccine project is to formulate a cost-effective, multistage, multicomponent malaria vaccine preparation that provides protection against the disease and can be employed in endemic areas most in need. In addition, the project will generally further the development of an urgently needed, versatile, human-compatible antigen delivery technology that can stimu-

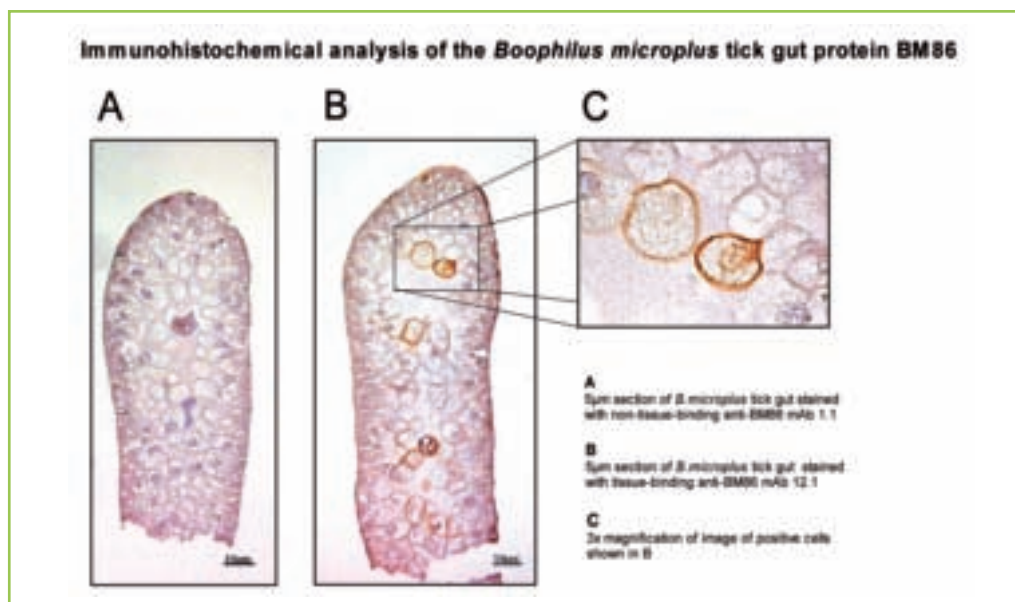
late different compartments and effector functions of the adaptive immune system.

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 Funding: EU, Commission for Technology and Innovation; Bundesamt für Berufsbildung und Technologie, Bern; Swiss National Science Foundation (SNSF)

3.2 Developing a second-generation anti-tick vaccine

Rapid development of acaricide resistance represents a significant threat to sustainable control of ticks and tick-borne diseases (TBDs) in livestock in tropical and subtropical countries. An optimal integrated TBD control programme is likely to include several approaches, such as the use of resistant breeds and vaccination, thereby allowing more strategic application of acaricides. The development of combined vaccines conferring protection against transmitted pathogens and the respective vectors may be a prerequisite for successfully developing vaccines against TBD.

We immunised a number of cattle with TickGARD, a commercial vaccine against *Boophilus microplus* based on a *B. microplus*-derived glycoprotein designated Bm86. This antigen represents a concealed protein presumably expressed on the surface of tick mid-gut cells. Immunised cattle were challenged with *B. decoloratus* ticks. Sera from cattle resistant to tick infestation were used to identify cross-reactive and potentially cross-protective



linear B cell epitopes conserved between *B. microplus* and *B. decoloratus*. A number of monoclonal antibodies (mAbs) were raised against a distinct linear epitope that is highly conserved among several tick species and that has also been described in independent studies as conferring some level of protection against *B. microplus* infestation. Tick-gut-binding and non-binding Bd86-specific mAbs have been established. The expression pattern of Bd86 throughout tick development and potential cross-reactivity with homologues of Bd86 expressed in other tick species has been studied in detail. Using in vitro membrane tick feeding systems, we will assess the tick inhibitory activity of these mAbs with the aim of determining in vitro correlates of protection.

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3.3 Synthetic carbohydrates as biomedical research tools and vaccine components

Polysaccharides are often very heterogeneous and difficult to isolate from their natural sources. Accordingly, recent improvements in carbohydrate synthesis technologies are paving new avenues for biomedical research and for vaccine design in particular.

Characterising *P. falciparum* glycosylphosphatidylinositol-specific antibody responses

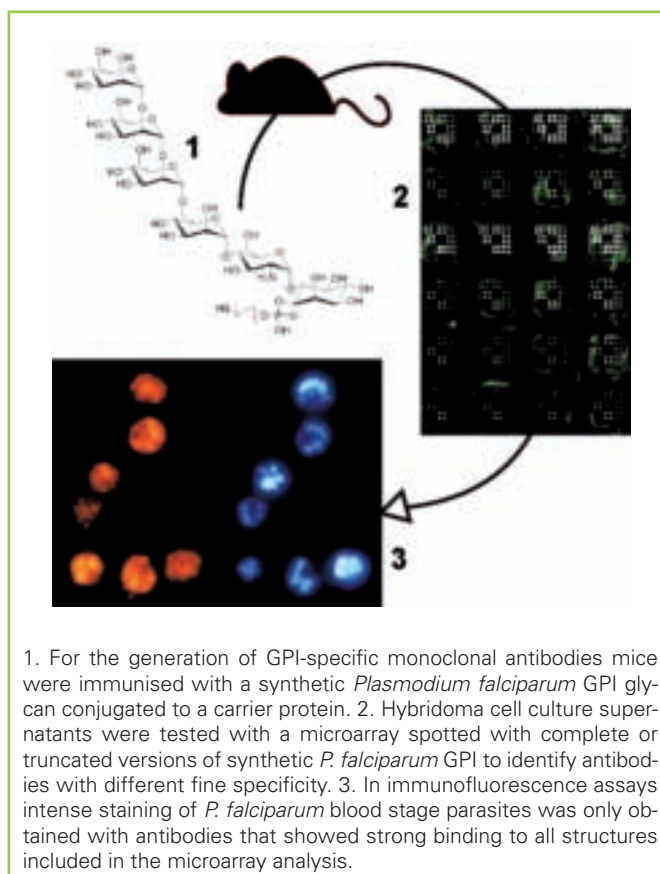
Glycosylphosphatidylinositols (GPIs) are ubiquitous molecules that serve as membrane anchors for certain proteins in eukaryotic cells. The core structure is highly conserved among eukaryotes. However, species-dependent branching confers structural specificities on GPIs of different origin. Plasmodial GPI is a potent activator of the host immune system and induces the secretion of so-called proinflammatory cytokines. These responses seem to play a critical role in the pathogenesis of severe malaria. Antibodies against plasmodial GPI have been postulated to mediate antitoxic and antidisease immunity against malaria, and GPI might be a suitable component for a malaria vaccine.

In collaboration with P. Seeberger (ETH Zurich) we have generated GPI-specific B cell hybridomas from mice immunised with a synthetic GPI glycan conjugated to a carrier protein. Cross-reactivity of the anti-GPI mAbs with GPI anchors of proteins of plasmodial and trypanosomal parasites was demonstrated by Western-blotting analysis. One of the six anti-GPI mAbs generated showed parasite-inhibitory activity in a *P. falciparum* blood-stage in vitro growth inhibition assay. In *Plasmodium berghei* and *Trypanosoma brucei rhodesiense* mouse infection models, passive immunisation with anti-GPI mAbs did not reduce parasite in vivo multiplication, and no delay of fatalities was observed. The mAbs generated represented new research tools that will help to characterise the GPI-anchored proteome of protozoan parasites.

Using a synthetic oligosaccharide to elicit *Bacillus anthracis*-specific antibodies

Recently, the structure of a tetrasaccharide carried by the exosporium glycoprotein BclA of *Bacillus anthracis* was elucidated. The tetrasaccharide consists of three rhamnose residues and a unique monosaccharide, anthrose. Since anthrose has not been described in other species, we envisioned detecting *B. anthracis* spores based on antibodies against anthrose-containing polysaccharides. Carbohydrate-protein conjugates containing the synthetic tetrasaccharide, an anthrose-rhamnose disaccharide and anthrose alone were synthesised by Seeberger's group and used to immunise mice. All three formulations were immunogenic and elicited IgG responses with different fine specificities. All sera and mAbs derived from tetrasaccharide-immunised mice cross-reacted with spores of a panel of virulent *B. anthracis* strains, but also with some closely related *B. cereus* strains. Although not strictly specific for *B. anthracis* spores, antibodies against the BclA-associated tetrasaccharide may have potential as immunocapturing components for a highly sensitive spore detection system.

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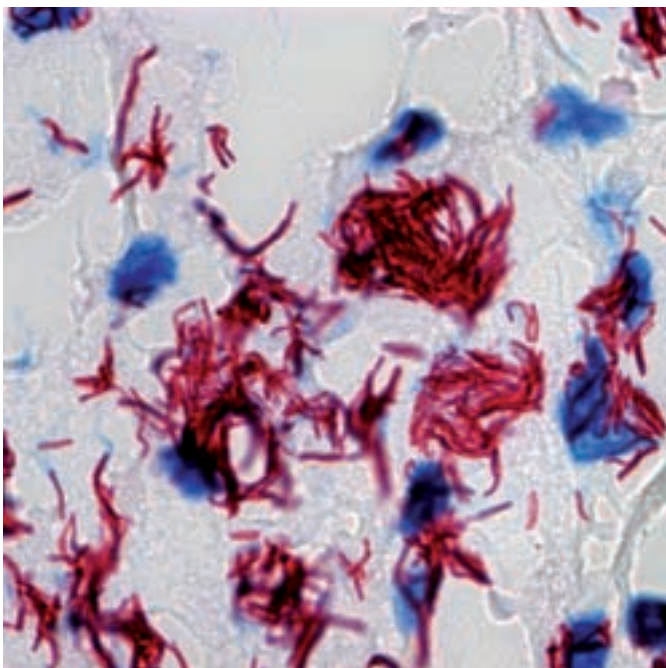


3.4 Meningococcal meningitis: clonal waves of colonisation and disease in the meningitis belt of sub-Saharan Africa

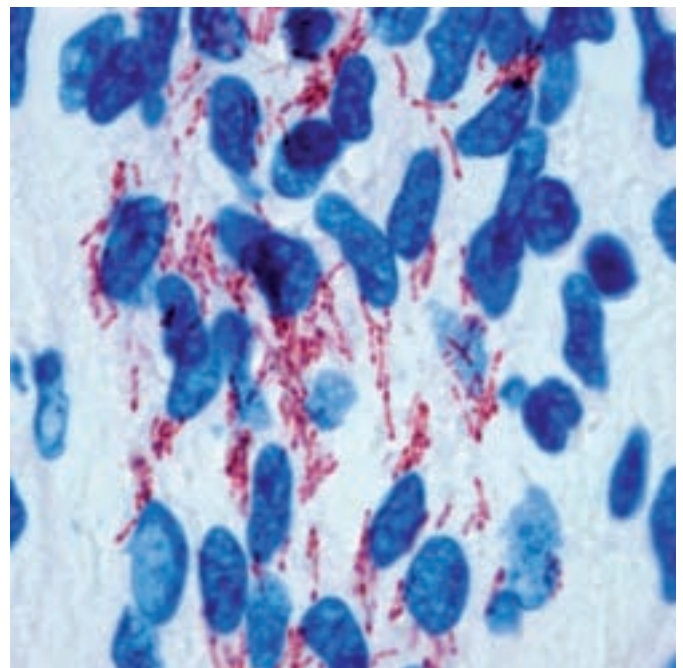
The highest burden of meningococcal disease occurs in the “meningitis belt” of sub-Saharan Africa. Within individual areas of this belt, major disease epidemics occur in irregular cycles every 8–12 years. Epidemics start in the early dry season, stop abruptly at the onset of the rains, but may break out again in the following dry season. In any one country, epidemics only last for 2–3 years. The periodicity of epidemics is not well understood, nor can they be accurately predicted. The meningococcus *Neisseria meningitidis*, a commensal of the human nasopharyngeal mucosa, is transmitted by aerosol droplets. Asymptomatic carriage is much more frequent than meningococcal disease. Epidemiological studies accompanying the introduction of a serogroup C meningococcal conjugate vaccine indicate that prevention of nasopharyngeal carriage and thus interruption of transmission plays a key role in preventing meningococcal disease in the vaccinated population. For this reason, studies on the impact of conjugate vaccines on carriage represent an essential element in the planned introduction of such vaccines in the meningitis belt. This prompted us in 1998 to initiate a long-term study of the dynamics of *N. meningitidis* carriage and disease in the Kassena-Nankana district (KND) of northern Ghana. This first longitudinal study of meningococcal colonisation in the sub-Saharan meningitis belt revealed features which in many aspects differ remarkably from research results in Europe and North America: the carried population of meningococci in the KND was (i) less genetically diverse, (ii) less constant in genotype composition, (iii) included fewer non-groupable strains and (iv) consisted mainly of

virulent encapsulated strains. Our data demonstrate that clonal waves of colonisation and disease are a characteristic feature of the meningitis belt. In the case of serogroup A, meningococcal genoclouds associated with the sequence types (STs) 5, 7 and 2859 have been responsible for the outbreaks in the last two decades. Our findings raise concerns that recent outbreaks caused by the serogroup A ST2859 genocloud in Ghana and Burkina Faso mark the beginning of a third wave of subgroup III meningococcal disease in the African meningitis belt.

A unique situation developed in the KND in 2006 with respect to meningococcal colonisation. While carriage rates during the 16 previous colonisation surveys had always been higher than the detection limit, not a single carrier of *N. meningitidis* was identified in the 2006 spring survey. When a new serogroup A clone associated with ST2859 appeared in 2007, colonisation rates increased dramatically (to 15.9% in November 2007). The worst-case scenario, emergence of a new hypervirulent clone and heavy colonisation of the population, thus had occurred, and there were major concerns that this would lead to a major epidemic. However, a dramatic drop in carriage – to 0.7% in April 2008 – followed, and in the meningitis season of 2008 only a few serogroup A ST2859 meningitis cases were observed in the KND. These data again emphasise the importance of carriage for development of disease outbreaks and epidemics. In collaboration with A. Sie (Nouna Health Research Centre, Burkina Faso) and T. Junghans (University Hospital Heidelberg), we have begun a similar longitudinal meningococcal disease and colonisation study in the Nouna health district of Burkina Faso. Here serogroup A ST2859 meningococci caused focal outbreaks in the northern part of the district. While >10% of the population of an outbreak village carried ST2859, the population in the



Clusters of *Mycobacterium ulcerans* multiplying in necrotic host tissue. (Photo T. Ruf)



After chemotherapy, dead *Mycobacterium ulcerans* cells with “beaded” appearance are found in Buruli ulcer lesions. (Photo T. Ruf)

southern part of the district was predominantly colonised by serogroup Y ST4375 meningococci, which were associated only with sporadic cases of meningitis. It will be very interesting to find out whether colonisation with the less-virulent Y meningococci will interfere with spread of the serogroup A ST2859 bacteria to the southern part of the district.

Population genomic studies have been initiated both for *Streptococcus pneumoniae* (genome sequencing of the hypervirulent serotype 1 pneumococcus clone responsible for an outbreak in Ghana) and *N. meningitidis* (comparative genomic sequencing of pools of colonisation and disease isolates), shedding light on our understanding of bacterial virulence and host-pathogen interactions in meningococcal meningitis. These studies make use of our large collection of epidemiologically very well defined bacterial strains.

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Funding: Volkswagen Foundation

3.5 *Mycobacterium ulcerans* infection (Buruli ulcer): towards improving control, diagnosis and therapy

Buruli ulcer (BU) caused by *Mycobacterium ulcerans* is considered to be the third most common mycobacterial infection after tuberculosis and leprosy. The disease has been reported in more than 30 countries worldwide, but children living in rural communities in sub-Saharan Africa are affected the worst. BU is a chronic necrotising skin disease mainly affecting subcutaneous and adipose tissue. The unique pathology of BU is primarily attributed to a plasmid-encoded macrolide toxin, mycolactone, which has cytopathic and apoptotic activity. Clinical lesions usually start as painless subcutaneous nodules that may develop into plaques or oedema. If left untreated, extensive



After surgical excision of Buruli ulcer lesions wound closure is achieved by autologous split skin grafting. (Photo G. Pluschke)

ulcerations with typical undermined edges of the dermis develop. While surgery has traditionally been the only recommended treatment for BU, in 2004 WHO published provisional guidelines recommending treatment with a combination of rifampicin and streptomycin for 8 weeks. In accordance with the priorities identified by the WHO Technical Advisory Group for BU, the goals of our research are to (i) improve understanding of the transmission of *M. ulcerans*, (ii) develop methods for early diagnosis, and (iii) investigate prospects for improving therapy and vaccine development.



Healed Buruli ulcer lesion associated with extensive scarring. (Photo G. Pluschke)

Transmission and genetic diversity of *M. ulcerans*

BU often occurs in focalised areas close to stagnant or slow-moving waters. The mode of transmission is not fully understood, partly because no molecular typing method has sufficiently high resolution for microepidemiological analyses. Our comparative genomic hybridisation analysis of *M. ulcerans* clinical isolates of diverse geographic origin revealed extensive large-sequence polymorphisms. The identified transposable element-associated insertional/deletional (InDel) recombination events are indicative of progressive genome shrinking in *M. ulcerans*, which emerged from the environmental mycobacterium *M. marinum* by acquiring a large virulence plasmid. Analysis of the large InDel polymorphism allowed us to distinguish between two distinct lineages: (i) the "classical" lineage representing the most pathogenic genotypes – those that come from Africa, Australia and South-East Asia; and (ii) an "ancestral" lineage comprising strains from China and Japan, South America and Mexico. Results suggest a preferential loss of immunodominant proteins, such as ESAT-6, CFP-10 and HspX. Since analysis of large-sequence polymorphisms did not differentiate between African isolates and because variable-number tandem repeat typing had only limited resolution, we are now focusing on identifying informative polymorphic single-nucleotide polymorphisms (SNPs). We have generated genome sequences of three *M. ulcerans* isolates. By comparing them to the only published genome sequence, several hundred SNPs were identified. We are currently evaluating the set of SNPs for their suitability for developing a genetic fingerprinting system for microepidemiological studies of BU.

Diagnosis

Inadequate laboratory resources in the highly disease-endemic areas of Africa often limit possibilities for in-country confirmation of clinical diagnoses. In a study in Ghana we found that a PCR test performed by an in-country reference laboratory is suitable for reconfirming clinical diagnoses. However, there is still a need for a

simple diagnostic test to aid fast pre-treatment confirmation of *M. ulcerans* infection at peripheral treatment centres in these areas. Our serological studies have indicated that exposure to *M. ulcerans* leads to clinical disease only in a minority of exposed individuals. Serological assays, such as one we developed based on 18-KDa Hsp, are useful for monitoring exposure to *M. ulcerans* but not for diagnosing BU. We therefore are now focusing on developing an antigen capture assay to be used in a point-of-care diagnostic test for BU.

Treatment

In our studies of lesions from BU patients treated with antibiotics, we have observed vigorous local immune responses and the development of ectopic lymphoid tissue. Results indicate that the relatively short antibiotic treatment reverses local immunosuppression and that the curative effect may be sustained by immune defence mechanisms. On the other hand, there is also growing evidence that some patients develop signs of immunopathology after antibiotic treatment.

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 Funding: Aide aux Lépreux Emmaüs–Suisse; Stanley-Thomas Johnson Foundation; Volkswagen Foundation

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