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# INTERNATIONAL STUDY GROUP ON NEW ANTIMICROBIAL STRATEGIES (ISGNAS): MEETING THE CHALLENGE OF RESISTANCE TO ANTIBIOTICS

THE ISGNAS WRITING COMMITTEE*

## SUMMARY

ISGNAS enables advancement of research through building a network of organisations and is also working to develop new antimicrobial strategies. Communication among participants is accomplished through published reports, E-mail, Internet, symposia, and special announcements.

## INTRODUCTION

The International Study Group on New Antimicrobial Strategies (ISGNAS) was formed in response to the recognition that development of microbial resistance to antibiotics is becoming a serious, world-wide problem. The group met in 1993 for the first time to discuss the feasibility of developing rational alternatives to the use of antibiotics and prepared as a result a comprehensive overview of normal (physiologic) mechanisms involved in the control of potentially pathogenic (opportunistic) microorganisms.

One objective of ISGNAS is to understand the conditions which allow opportunistic microbes present among the symbionts to cause an infection. There is a need for more coherent information concerning the habitat, growth requirements and host and pathogen properties which allow opportunistic pathogens to cause life-threatening infections. In particular, information is urgently being sought to understand the complexity of the interactions between the vast number of microbial species, and the interactions between the microbes and their host.

Another goal is to inspire and enable basic and clinical research that will lead to the development of new therapies for regulating colonisation, translocation and infection by opportunistic microorganisms in patients during periods of decreased resistance. With a sufficient amount of knowledge of how healthy individuals keep opportunistic microorganisms under control, it may become feasible to iatrogenically maintain host-resistance and inter-microbial factors involved in containment of opportunistic microbes. Therapies aimed at bolstering natural resistance mechanisms will be of critical importance to individuals whose resistance has been compromised as a result of another clinical condition.

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## CONCLUSIONS AND RESEARCH RECOMMENDATIONS BASED ON THE ISGNAS REVIEW

It was decided that the information in the review of ISGNAS would be used to identify gaps in the present knowledge. The subjects described in the gaps are described by ISGNAS as Research Priorities. These research priorities would serve as a lead for research in experimental animals as well as in man (patients) to be conducted by experts in well experienced centres in the world. The following paragraphs are based upon the ISGNAS review (*Areneo* et al., 1996).

### I. The normal gastro-intestinal microflora (GIM)

Bacteria, resident in the intestinal microflora suppress multiplication of newly ingested bacteria and may (sterically) hinder their adhesion to the mucosal lining. Accordingly, ingested bacteria and yeasts either die or get gradually eliminated by excretion with the faeces. This first line of defence is called Colonisation Resistance (CR).

CR flora may release substances which interact/interfere with receptors and adhesion molecules for bacteria on host cells. They could also release/produce immunomodulating substances. This modulation obviously occurs predominantly in the 'gut associated part' but may also modulate the 'systemic part' of the immune system, the mononuclear phagocytosing system (MPS) and the bone marrow. CR flora may also interact with the neuro-endocrine system and condition the mucosal or epithelial barrier. Finally, the composition of the diet influences the actual composition of the intestinal microflora.

Research Priorities:

- Development of technique(s) to accurately determine the composition of the GIM and the quality of the CR in a short time and at low costs.
- Study of substances (nutritional and microbial) by intestinal microorganisms involved in interaction with receptors for bacterial adhesion on mucosal cells.
- Study of bacteria in the normal microflora which produce immuno-modulating substances, to chemically characterise the chemical composition of these substances and to identify target host cells and their responses.
- Study of indigenous and exogenous microbial substances which influence bone marrow haemopoietic activity.
- Study of the influence of the composition of the diet and probiotics on the microflora and on the host.
- Study of substances which influence the immuno-neuro-endocrine system.
- Study of substances which influence the GI-tract motility.

### II. Intestinal barrier integrity

*In vivo* loss of intestinal barrier integrity has been reported in the myriad of clinical conditions noted to predispose a patient to bacterial translocation. Essentially, all the diverse clinical conditions are associated with bacterial translocation and nearly all these conditions are associated with altered intestinal flora, which mostly concerns bacterial overgrowth. Increased intestinal permeability (opening of epithelial occluding junctions) can clinically be assessed by several methods including urinary excretion of orally administered agents.

Research Priorities:

- The mechanism whereby loss of intestinal barrier function might permit intestinal bacteria to migrate out of the intestinal lumen to distal sites.

## III. Microbial interactions with the intestinal mucosa

The mucosa produces a mucus layer which is a defence mechanism. The mucosa, under normal physiological conditions, would allow translocation of microorganisms to a limited extent.

The microflora stimulates (perhaps indirectly) the activity of the undifferentiated cells in the crypts of the intestinal mucosa.

Research Priorities:

- Identification of the [type(s) of] microorganisms and of [the type(s) of] host cells involved in the modulation of mucus production by mucosal cells (modulation of the quality of the feeder layer).
- Study of bacteria in the intestinal tract which modulate proliferation and maturation of undifferentiated crypt cells.
- Study of the process of translocation of bacteria as well as their bio-active fragments.

## IV. Site of clearance of microorganisms as well as microbial fragments

The gut lamina propria, the mesenteric lymph nodes, the spleen, the liver and the bone marrow can be regarded as important clearance sites. In the lamina propria translocated microorganisms might be cleared in a non-inflammatory way. This system may be functioning even under normal physiological conditions. The liver, being directly supplied with blood from the intestines, is in an exceptional position and may be primed for the clearance of bacteria and bacterial products in the systemic circulation. Modulation of the GI-tract microflora may also influence the function of hepatocytes.

Research Priorities:

- To investigate whether the lamina propria is an important site at which a significant proportion of translocated bacteria and yeasts are cleared.
- To identify factors to stimulate the production of specific and non-specific opsonisation of translocated microorganisms and microbial fragments.
- To identify factors by which the intestinal microflora interacts with the function of hepatic cells.
- To identify factors which increase the systemic clearance capacity.

## V. The gut associated lymphoid tissues (GALT) and the systemic immune system

The GALT includes lymphoid organs in the GI-tract (Peyer's patches), lymphocytes in the lamina propria. The systemic immune system involves the thymus, the bone marrow and the lymph nodes (not associated with the gut) and the spleen.

Research Priorities:

- Study of cellular and humoral immune responses which are induced by normal microflora as well as the induction sites normally used by bacteria. This includes opportunistic bacteria and yeasts as well as non-pathogenic microbes.
- Study of the implication of the type of immune response induced for the development of inflammatory (clinical) signs and symptoms. The relevance of the different isotypes of antibodies produced.
- Elicit the roles and function of IgA.
- Determine the importance of oral tolerance.
- Role of the immune system on the composition of the microflora (its modulating capacity and any possible replacements).
- Identification of members of the microflora in the development and activation of the mucosal immune system.
- Study of the effectiveness of vaccination; active or passive.

- Identification of factors released by the microflora which are chemotactic to phagocytic cells.
- Evaluate the efficacy of immuno-modulators in increasing both systemic and mucosal non-specific immunity to pathogens.

### VI. The bone marrow

The bone marrow is the site of origin of, among else, specific and non-specific immune cells.

Research Priorities:

- To identify factors which may focus haemopoietic activity towards cells involved in the non-specific immune system with the purpose of eventually manipulating the system.
- To study the influence of flora modulation and other factors on the survival of stem cells (bone marrow as well as intestinal crypts cells) during chemotherapy/irradiation.

### VII. Immuno-neuro-endocrine system

The Immuno-neuro-endocrine system is under influence of the gut microflora and vice versa, and controls on the other hand also the systemic resistance.

Research Priorities:

- To identify factors released by the microflora which locally or systemically modulate the neuro-endocrine system.
- To modulate the neuro-endocrine system to maximally control opportunistic pathogens.

### VIII. Mathematical modelling of the host-microflora interaction

As new detection methods are developed and observational data on the gastro-intestinal microflora (GIM) and its interaction(s) with the host are improved, it will become increasingly necessary to develop a theoretical framework to explain the observations. As mathematical models have been used successfully in a number of different ecosystems, both microbial and of higher organisms, it is not unreasonable to assume that such models may increase our insight in this case. Especially in the case of complicated, non-linear systems (including the immune system), computer simulation can provide a powerful means to integrate knowledge obtained from studying various parts of the system in isolation, and to provide insights into the dynamics. Advanced time series analysis techniques on data obtained by other ISGNAS efforts must be used to validate the models.

Research Priorities:

- Development of time series analysis techniques to detect the presence of non-linear (chaotic) behaviour in the dynamics of the GIM and the defence system.
- Development of a computer model of the GIM based on bacterial physiology; comparison of theoretical and observed dynamics.
- Integration of the above model with models of the immune system, bowel motility etc. developed elsewhere (or currently under development).

## RESOURCES OF ISGNAS

The international study group consists of biomedical scientists (both medical and basic), each an expert in a different subspecialty of host defence mechanisms (Table 1).

**Table 1**: Membership of ISGNAS

| Member | Location | Expertise |
|---|---|---|
| J. Beuth | Köln, Germany | Bacterial immune stimulating products |
| J. Bienenstock | Hamilton, Canada | Neuro-immuno-endocrinology |
| J.J. Cebra | Philadelphia, USA | Intestinal immunology |
| P.J. Heidt | Rijswijk, The Netherlands | Gnotobiology |
| W.L. Manson | Groningen, The Netherlands | Infections in patients with decreased resistance |
| T. Midtvedt | Stockholm, Sweden | Microflora acquired characteristics (MACs) and Germfree associated characteristics (GACs) |
| J.P.T.M. Noordhuizen | Wageningen, The Netherlands | Zootechnology/Infectious disease control in agriculture |
| C.E. Nord | Stockholm, Sweden | Antibiotic resistance |
| P. Nieuwenhuis | Groningen, The Netherlands | Systemic immunity |
| V. Rusch | Herborn, Germany | Immune stimulation by live bacteria |
| D. van der Waaij | Groningen, The Netherlands | Medical microbiology |
| R.I. Walker | Rockville, USA | Vaccines |
| M.H.F. Wilkinson | Groningen, The Netherlands | Mathematical modelling |

## LITERATURE

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