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NSG mouse strain, health monitoring and microbiological findings

J Sparrowe[1], L Jiménez[1], A Talavante[1], I Angulo[2], A Martínez[1]

[1]Laboratory Animal Science, [2]Malaria Therapeutic Efficacy
Diseases of the Developing World-Drug Discovery Centre. GSK R&D. Severo Ochoa, 2. 28760 Tres Cantos, Madrid, Spain.

**Correspondence: John Sparrowe, GSK R&D S.L., Diseases of the Developing World-Drug Discovery Centre*
P.T.M Severo Ochoa, 2 - 28760. Tres Cantos, Madrid, Spain
Mobile *+34 609 570 874*
Tel *+34 91 80 70653*
Email *john.g.sparrowe@gsk.com*

Summary

The NSG strain is one of the most immunodeficient mice available, and provides an effective model for studies where the engraftment of human cells is required. However because of their severe adaptive and innate immune deficiency, these mice must be kept under high environmental control standards.

Routine health monitoring, according to FELASA guidelines, included antigen testing in NSG animals and antibody testing in sentinel animals (negative results, not shown).

We found saprophytic flora, by use of classic microbiology techniques, in a variety of tissues and organs. Many of the sampled animals were found to have bacteria growing in the spinal cord, tarsal joint, heart, spleen, liver, kidney or blood. In order to rule out possible tissue contamination, we sent samples to three different external laboratories. All the samples submitted came back with the same results.

We postulate that the widespread presence of these saprophytic bacteria may be due to a lack of IgA secretion at the mucosal epithelium, and the bacterial growth in these tissues and organs to the immunodeficiency including impaired macrophage activity. The potential clinical significance of these bacteria in NSG mice, if any, is not known. To explore the possible connection between the bacterial infection and animals with signs of slight limb paresthesia (numbness) and paralysis, and arthritis, seen in 0.5-1% of animals, further studies are needed.

Introduction

NSG mice are severely immunocompromised animals. Their main features are absence of mature T or B cells, lack of functional NK cells and deficiency in cytokine signaling. Immunological cells present in these immunodeficient mice are neutrophils, monocytes, macrophages and dendritic cells, though the last two are hypofunctional (Schultz *et al.*, 2005).

The NSG mouse strain provides an invaluable tool to accelerate the development of potential therapies for the treatment of malaria in a Humanized Mouse Model of *Plasmodium falciparum* malaria at our Institution (Jiménez-Díaz *et al.*, 2009).

Despite the high immunodeficiency of these mice, when maintained appropriately they do not show health problems. The only clinical sign that we have observed has been a mild paresthesia with paresis of the digits in both the fore and hind limbs, and associated arthritis, in 0.5-1% of the animals. These clinical signs were detected upon the animals' arrival at our laboratory (Figure 1).

Material and methods

Animals

NSG (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/*SzJ)* mice were purchased from Jackson Laboratories and Charles

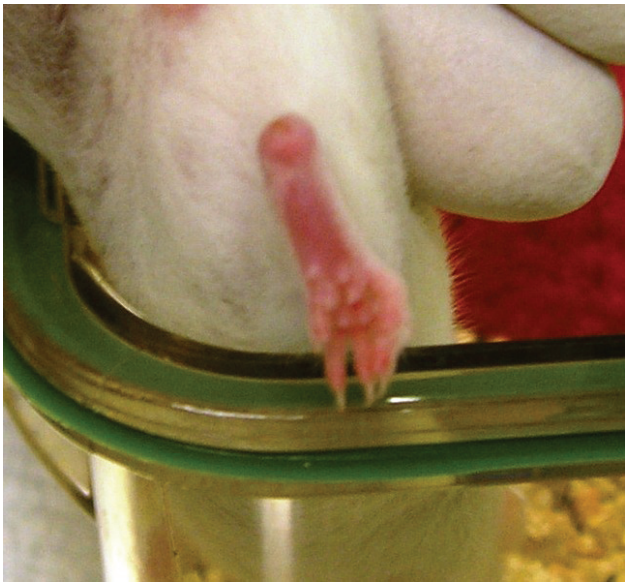

Figure 1. Right tarsal joint arthritis

River Laboratories, and were maintained under maximum barrier conditions.

All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals.

Health Monitoring

Routine health monitoring of the NSG strain was performed on 43 naive NSG mice. These were randomly selected on arrival, with uncrating of the boxes within a Class II biological safety cabinet, in a laboratory external to our facility. After housing the animals inside our facility, seven mice showing swollen tarsal joints and/or gait alterations were immediately isolated and also underwent the same health monitoring procedure within the following 24 hours. The program included routine extended FELASA profile, with necropsies, and detection of pathogens' antigens including pathogenic bacteria by PCR, RT-PCR and culture.

NSG strain health was also screened by classic microbiological culture of samples, aseptically taken, and individually processed and cultured, from: oral cavity and cecum by swabbing, spinal cord by blowing the vertebral canal using sterile syringes, and from lung, heart, liver, kidney, spleen, tarsal joint and blood. Samples were checked for the following opportunistic and commensal agents: Streptococcus α –haemolyticus, Enterococcus, coagulase positive and negative Staphylococcus, *Lactobacillus spp*, *Corynebacterium spp,* Coliform LFC's (*E.coli*, *Klebsiella spp*, *Enterobacter spp*, *Citrobacter spp*…), Coliform NLFC's (*Proteus spp*, *Serratia*...), and water transmitted microorganisms (*Pseudomonas spp, Alcaligenes spp*, *Acinetobacter spp*).

Necropsies were performed at the Madrid School of Veterinary Medicine, Universidad Complutense. Swabs and samples were kept in AMIES transport medium at 4 °C and were sent refrigerated at 4-8 °C to three independent international diagnostic laboratories that used API VITECK 2 - a system designed primarily to identify human and animal bacterial pathogens - for bacterial growth, isolation of primary cultures and identification.

Results

Healthy animals (control group)

All animals were negative for the FELASA list of pathogens. Regarding the microbiological culture work looking for saprophytic or opportunistic agents, the results of the three external laboratories were in concordance. Growth of many commensal bacterial strains was found in almost all tissues sampled, in a high percentage of the animals, without a predominant bacterial strain pattern. The most frequent location with growth was the spinal cord.

List of microorganisms isolated in each tissue in order of frequency:

Spinal cord: *E. coli* (7 mice), *Strep. mitis* (5 mice), *Staph. coag. neg.* (5 mice), *Lactobacillus spp* (4 mice), *A. viridans* (3 mice), *Staph. aureus* (2 mice), *E. faecalis* (2 mice), *E. cloacae* (1 mouse) and *E. faecium* (1 mouse); lungs: *E. coli* (1 mouse) and *Lactobacillus spp* (1 mouse); heart: *E. faecalis* (4 mice), *Lactobacillus spp* (2 mice), *Staph. aureus* (1 mouse), *E. coli* (1 mouse) and *Strep. mitis* (1 mouse); liver: *Strep mitis* (3 mice), *Lactobacillus spp* (3 mice), *Staph. aureus* (1 mouse), *A. viridans* (1 mouse) and *E. faecalis* (1 mouse); kidney*: Lactobacillus* spp (5 mice), *E. faecalis* (3 mice*), Staph. coag. neg.* (3 mice), *A. viridans* (2 mice) and *E. coli* (2 mice); spleen: *E. coli* (3 mice), *Lactobacillus spp* (2 mice), *Staph. aureus* (1 mouse), *Strep. mitis* (1 mouse), *E. faecium* (1 mouse) and *E. faecalis* (1 mouse); tarsal joint: *E. coli* (2 mice), *Strep. mitis* (1 mouse), *E. faecium* (1 mouse) and *Lactobacillus spp* (1 mouse); blood: *Staph. aureus* (1 mouse), *Strep. mitis* (1 mouse), *E. faecalis* (1 mouse) and *Staph. coag. neg.* (1 mouse).

The histopathology report also stated that there were multifocal and marked bacteria adherent to the mucosal surface in cecum and colon of all the animals (Figure 2).

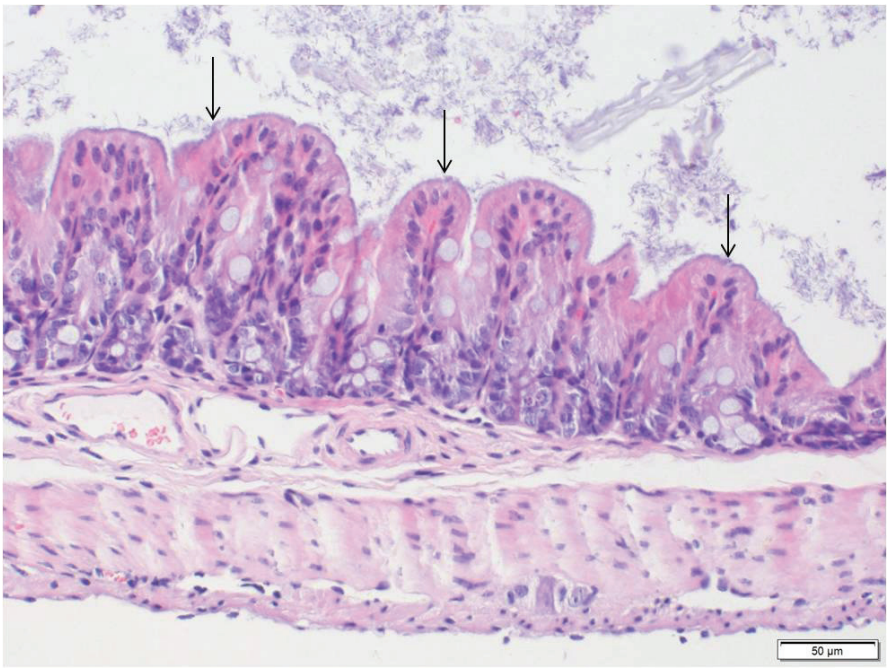

Figure 2. Bacteria adherent to the mucosal surface of colon, multifocal, marked.

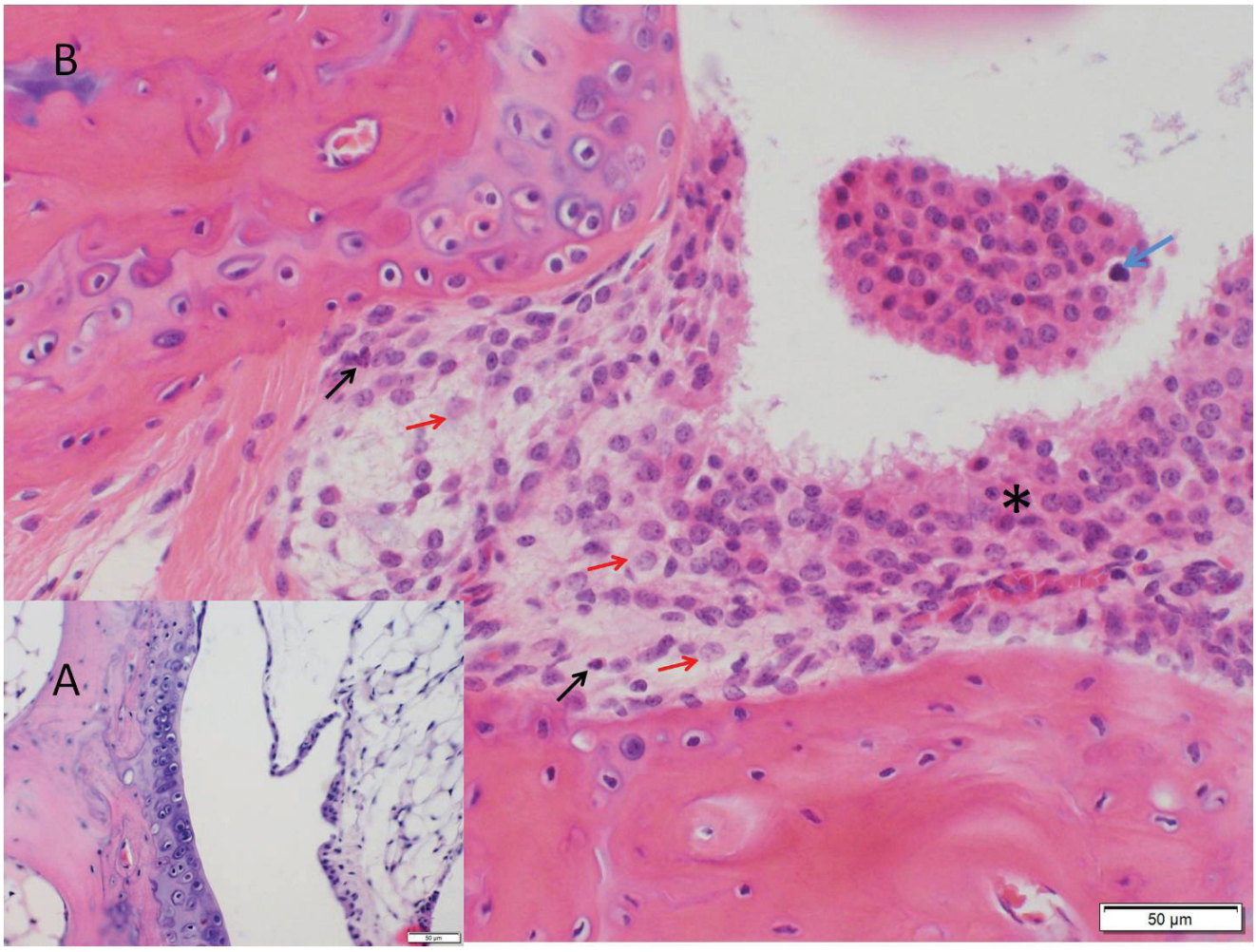

Figure 3. A. Normal tarsal joint. B. Arthritis, chronic, moderate, presence of inflammatory cells: neutrophils (black arrows) and macrophages (red arrows), nuclear pyknosis (blue arrow) and hyperplastic synovial lining (*)

Animals with clinical signs

The same procedure was performed on seven animals showing slight limb numbness and paralysis and arthritis. The outcome was different, as bacteria were found less frequently, particularly in the spinal cord.

List of microorganisms isolated in each tissue in order of frequency:

Spinal cord: *E. coli* (1 mouse), *Strep. mitis* (1 mouse) and *G. morbillorum* (1 mouse); these three findings refer to the same individual; liver: *G. morbillorum* (1 mouse); spleen: *G. morbillorum* (1 mouse); all the other tissues were culture negative. The pathologist also found arthritis in the tarsal joint or thickening of joint capsule (Figure 3), and the same observations in cecum and colon mucosa.

The percentage of mice with bacterial growth in various organs was higher in NSG mice without clinical signs (control group), see Figure 4. The percentage of mice with bacteria in the spinal cord was particularly high in the control group.

Discussion

All the bacterial strains found in tissues were also isolated and identified in cecum and/or oral cavity. These bacterial strains can be considered commensal or opportunists. One of the opportunists found was *Staph. aureus*. It was isolated from spinal cord, heart, liver and spleen, identified by molecular detection, and sequenced for confirmation in three control animals of the same batch, but only in one out of a large number of shipments. Although pathogenicity could be expected due to the many potential virulence factors of *Staph. aureus*, no differences were shown in the health of NSG mice from the same group. . The list of organisms monitored at the supplier's breeding colony, as shown in their animal health reports under the "other organisms monitored" section, includes *Staphylococcus aureus*. This "other agents" monitoring agrees with the FELASA recommendations for the health monitoring of immunodeficient animals (Mähler *et al.*, 2014); it indicates the necessity to monitor such animals for opportunists or commensals, but also acknowledges that to define a complete list is impossible.

Although the reason for the bacterial colonization of the tissues of NSG mice is understandable, it is unclear whether it should be considered an infection. The absence of functional B cells implies a zero level of immunoglobulin. Thus mucosal barriers do not have IgA dimers (Foreman *et al.*, 2011) in mucosal secretions to control the spread of bacteria across this barrier.

These mice also lack NK cells and T lymphocytes, so survival of invading microorganisms is favoured. The presence of bacteria in normally sterile body sites did not correlate with inflammation. Despite the serious immunological impairment, these mice did not show morbidity, other than a few cases of mild arthritis. Their good health can be explained by the non pathogenic nature of the saprophytic/opportunistic bacteria and the absence of a host reaction.

There seems to be some natural tropism of bacteria for spinal cord tissue in the control group, with a much higher incidence of bacteria in the spinal cord than in the mice showing signs of arthritis/paralysis.

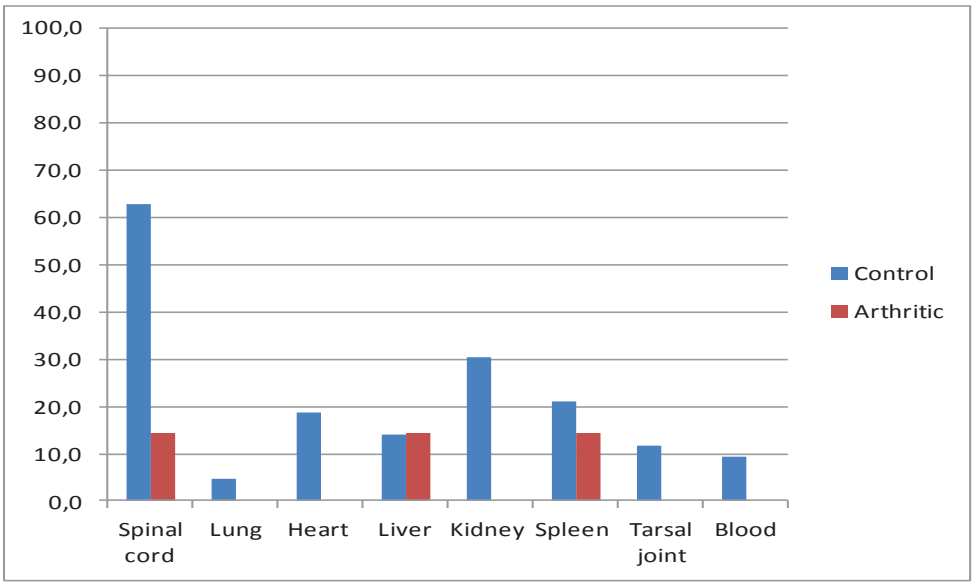

Figure 4. Percentage of NSG mice with bacteria in various organs and tissues.

It can be hypothesized that, although immunologically impaired, it is likely that phagocytic activity was involved in eliminating bacteria from the mice with clinical signs (Hu *et al.*, 2011). It is possible that increased levels of Il-6 and other proinflammatory cytokines, secreted by phagocytes and by dendritic cells involved in killing the bacteria might explain the clinical signs seen in these animals (Liang *et al.*, 2009).

Conclusions

The addition of microbiological culture of tissues to the health monitoring routine for immunodeficient strains showed that the normal condition of the NSG strain, when they arrive at our facility, is to contain some amount of normal flora and various opportunistic bacterial strains disseminated throughout their bodies, despite a low incidence of clinical signs or lesions found in tissues.

To explore the possible connection between these bacteria, an altered immune response to them and animals with signs of slight limb numbness and paralysis, and arthritis, further studies are needed.

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