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Guillain Barré Syndrome is induced in Non-Obese Diabetic (NOD) mice following *Campylobacter jejuni* infection and is exacerbated by antibiotics

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ABSTRACT

Campylobacter jejuni is a leading cause of bacterial gastroenteritis linked to several serious autoimmune sequelae such as the peripheral neuropathies Guillain Barré syndrome (GBS) and Miller Fisher syndrome (MFS). We hypothesized that GBS and MFS can result in NOD wild type (WT) mice or their congenic interleukin (IL)-10 or B7-2 knockouts secondary to *C. jejuni* infection. Mice were gavaged orally with *C. jejuni* strains HB93-13 and 260.94 from patients with GBS or CF93-6 from a patient with MFS and assessed for clinical neurological signs and phenotypes, anti-ganglioside antibodies, and cellular infiltrates and lesions in gut and peripheral nerve tissues. Significant increases in autoantibodies against single gangliosides (GM1, GQ1b, GD1a) occurred in infected NOD mice of all genotypes, although the isotypes varied (NOD WT had IgG1, IgG3; NOD B7-2$^{-/-}$ had IgG3; NOD IL-10$^{-/-}$ had IgG1, IgG3, IgG2a). Infected NOD WT and NOD IL-10$^{-/-}$ mice also produced anti-ganglioside antibodies of the IgG1 isotype directed against a mixture of GM1/GQ1b gangliosides. Phenotypic tests showed significant differences between treatment groups of all mouse genotypes. Peripheral nerve lesions with macrophage infiltrates were significantly increased in infected mice of NOD WT and IL-10$^{-/-}$ genotypes compared to sham-inoculated controls, while lesions with T cell infiltrates were significantly increased in infected mice of the NOD B7-2$^{-/-}$ genotype compared to sham-inoculated controls. In both infected and sham inoculated NOD IL-10$^{-/-}$ mice, antibiotic treatment exacerbated neurological signs, lesions and the amount and number of different isotypes of antiganglioside autoantibodies produced. Thus, inducible mouse models of post-*C. jejuni* GBS are feasible and can be characterized based on evaluation of three factors—onset of GBS clinical signs/phenotypes, anti-ganglioside autoantibodies and nerve lesions. Based on these factors we characterized 1) NOD B-7$^{-/-}$ mice as an acute inflammatory demyelinating polyneuropathy (AIDP)-like model, 2) NOD IL-10$^{-/-}$ mice as an acute motor axonal neuropathy (AMAN)-like model best employed over a limited time frame, and 3) NOD WT mice as an AMAN model with mild clinical signs and lesions. Taken together these data demonstrate that *C. jejuni* strain genotype, host genotype and antibiotic treatment affect GBS disease outcomes in mice and that many disease phenotypes are possible.

1. Introduction

Guillain Barré Syndrome (GBS) is an autoimmune post-infectious polyradiculo-neuropathy characterized by limb weakness and loss of tendon reflex [1]. GBS is a monophasic disease, with the most severe disease occurring 2–4 weeks after first onset of symptoms; yet 7% of GBS patients have reported reoccurrence [2,3]. Weakness is followed rapidly by symmetrical ascending paralysis which can progress to paralysis of the muscles of respiration. Males are more likely to develop GBS than females with a ratio of 1.25–1.5 males to 1.0 females [1,4]. Current treatments for GBS include

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plasma exchange and intravenous immunoglobulin [2,4]. Despite treatment 10–20% of patients do not recover completely, many suffer life-long disability and the mortality rate is 3–10% [3].

GBS is defined as a group of disorders differentiated by particular forms of immune attack on the peripheral nervous system. Subtypes of GBS include acute inflammatory demyelinating polyneuropathy (AIDP), acute motor axonal neuropathy (AMAN), and Miller Fisher Syndrome (MFS). AIDP is the most common subtype; occurring in approximately 90% of GBS cases in North America and Europe [3,5]. AIDP presents as inflammatory segmental demyelination affecting mainly myelinated nerves of the limbs and lower cranial and sensory nerves. AMAN is a form of GBS seen mainly in China, Japan, and Central and South America with a frequency of 38–65% [5]. AMAN patients present with damage to the motor neurons at the nodes of Ranvier. A related form—acute motor sensory axonal neuropathy (AMSAN) — causes dysfunction of peripheral motor and sensory nerves [6]. MFS is a variant of GBS accounting for 5% of all GBS cases worldwide [7], while in eastern Asia, MFS occurs at a rate of 20–25% [3,5]. This anatomically localized variant of GBS affects mainly the cranial nerves causing areflexia, ataxia, and ophthalmoplegia [8,9]. Unlike GBS, MFS causes descending paralysis, but can progress to ascending paralysis [10] particularly if axonal degeneration occurs [1]. Motor strength is usually not affected, and recovery is generally gradual but complete [1].

Two-thirds of GBS patients have a history of prior respiratory or gastrointestinal infections [1,3,4,11]. In 1993, Mishu and Blaser recognized that infection with *C. jejuni* can result in development of GBS [12,13]. Now known antecedent infections for GBS include the bacterial and viral agents *Campylobacter jejuni, Mycoplasma pneumoniae,* Epstein-Barr virus, and cytomegalovirus [1–3,14–16]. *C. jejuni* is the most common enteric infection associated with development of the AMAN form of GBS worldwide and is the most frequent bacterial etiology for human gastroenteritis with 1–2.4 million cases annually in the U.S [3,11,17–19]. The risk of developing GBS after *C. jejuni* infection is less than 1 GBS case per 1000 *C. jejuni* infections in the U.S. [2,20,21], but cases and outbreaks occur worldwide. Neurological symptoms of the AMAN subtype occur 1–3 weeks after the onset of *C. jejuni* induced enteric disease, although some of these GBS patients are colonized without enteritis [3,14].

In GBS, molecular mimicry is thought to play a role in the production of anti-ganglioside antibodies, which bind to and trigger attack on host nerve tissue [2,5,11,14,17,19,22–25]. The structure of lipooligosaccharides (LOS) of *C. jejuni* can mimic nerve gangliosides in the host, eliciting an immune response that cross-reacts with gangliosides in the peripheral nerves [2,26]. It has been shown both in humans and in animal models that the immune response to the bacterium results in production of anti-ganglioside antibodies that leads to damage of nerves and Wallerian-like degeneration; such “autoantibodies” have been shown to bind to motor neurons, nodes of Ranvier, and neuromuscular junctions [21,26–29]. Autoantibodies directed against gangliosides GM1 and GD1a have been detected most commonly in patients with the AMAN variant of GBS, while autoantibodies against GQ1b are more often associated with MFS [30–32]. The peak titers of anti-ganglioside antibodies following *C. jejuni* infection have been associated with onset of GBS symptoms in patients. An example of correlation between autoantibodies and disease manifestations occurred during an outbreak of *C. jejuni* in 2007 when 36 cases of GBS were identified in northern China in patients that produced high titers of anti-GM1 autoantibodies. Bacterial fecal culture was performed, and *C. jejuni* was identified as the antecedent infectious agent [33]. Another study conducted in southeast England during 1983–1984 examined serum samples of 95 GBS patients and 88 control patients demonstrating that 14/95 patients had high titers of antibodies reactive to GM1 based on ELISA testing [34].

Despite these advances, progress in understanding the pathogenesis of GBS and in developing effective therapies has been slowed due to the lack of tractable animal models. Currently, rabbits and chickens serve as induced animal models for GBS. GBS is induced in the rabbit model by immunizing rabbits with *C. jejuni* LOS known to mimic particular gangliosides. Rabbits given 2.5 mg *C. jejuni* LOS subcutaneously at 3 week intervals developed flaccid limb weakness between 133 and 329 days after the initial inoculation, while rabbits treated similarly with 10 mg LOS developed tetraparesis 40–227 days after the initial inoculation [19]. Anti-GM1 IgM and anti-GM1 IgG antibodies were detected 2–4 weeks and 4–6 weeks, respectively, after the first injections [19,35]. Although LOS exposure elicits anti-ganglioside reactivity, immunized rabbits are not a precise model for GBS induced following oral *C. jejuni* infection because humans are exposed through a foodborne route. Alternatively, chickens acquire a form of peripheral neuropathy secondary to oral inoculation with *C. jejuni* GBS patient strains. Thirty-three percent of chickens receiving *C. jejuni* HB93-13 orally became paralyzed within 12 days [36]. In paralyzed chickens, early lesions included nodal lengthening and paranodal demyelination that was followed by Wallerian-like degeneration and even paranodal re-myelination in some long-term survivors. Thus, chickens inoculated orally with GBS associated *C. jejuni* strains from patients can be considered a naturally occurring model for GBS. However, chickens are outbred and very different anatomically and physiologically from humans, which detracts from their value as a model. Susceptible inbred mice would provide the ideal model where the interplay of host and pathogen genetics could be explored.

Our laboratory has shown that C57BL/6 IL-10$^{+/+}$ and congenic IL-10$^{-/-}$ mice can serve as *C. jejuni* colonization and colitis models, respectively [37,38]. In response to a colitogenic isolate of *C. jejuni* (11168), both innate lymphoid cells (ILCs) and T cells participated in interferon-γ (IFN-γ), IL-17, and IL-22 upregulation in a time- and organ-specific manner. Depleting IFNγ, IL-17 or both significantly ameliorated colitis and drove colonic responses toward type 2 cytokine and antibody induction. Thereafter, we demonstrated that *C. jejuni* GBS patient strains (HB93-13, 260.94) induced mild colitis associated with blunted T helper 1/17 but enhanced T helper 2 adaptive immune responses in orally infected C57BL/6 IL-10$^{-/-}$ mice [39]. Moreover, the type 2 but not type 1/17 antibodies cross-reacted with peripheral nerve gangliosides, demonstrating autoimmunity. In mice infected with the GBS strains but not the colitogenic strains, autoreactive antibodies were exclusively of the IgG1 isotype. Thus we concluded that molecular mimicry and not just the cytokine milieu was essential for autoantibody development because the colitogenic non-GBS strain *C. jejuni* 11168 failed to induce auto-antibodies even though it expresses a GM1 mimic and the immune response was biased toward type 2 after depleting IFNγ and IL-17.

Non-Obese Diabetic (NOD) inbred mice have been documented to develop autoimmune diabetes mediated by auto-reactive T cells infiltrating the pancreas [40]. They also develop autoimmune diseases of the salivary glands and thymus [30]. Additionally, NOD mice deficient for the co-stimulatory molecule B7-2 (NOD B7-2$^{-/-}$ mice) are largely protected from autoimmune diabetes but develop a spontaneous autoimmune peripheral polyneuropathy called chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) that resembles GBS [30,41]. By 20 weeks of age, peripheral nerves of neuropathic NOD-B7-2$^{-/-}$ mice have infiltrates of dendritic cells and CD4^{+} and CD8^{+} positive T cells. Based on knockout of genes in mice of the same genetic background, these investigators showed that neuropathy developed in the absence of