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Rat Model for Poly-Autoimmunity

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ABSTRACT

Around 30% of the male PVG rats have very low testosterone levels. Upon immunisation with myelin basic protein in complete Freund's adjuvant they develop experimental autoimmune encephalomyelitis and adjuvant arthritis. The androgen insensitive syndrome in these rats could be used in studies on poly-autoimmunity as well as in studies on variations in the sexual differentiation of brain and behavior.

Keywords: Male PVG Rat, Experimental Autoimmune Encephalomyelitis, Adjuvant Arthritis, Poly-Autoimmunity, Sexual Behavior

1. INTRODUCTION

There is vast literature on neuro-immuno-endocrynology with excellent reviews on multiple-autoimmunity, i.e., the existence of several autoimmune diseases (Bergsteinsdottir *et al*., 2000; Rojas-Villarraga *et al*., 2012; NNE, 2012). The numerous studies describing different parts in the hypothalamic-pituitary-adrenal axis and the interplay between the gonadal steroids and the immune system, with respect to autoimmunity in the central nervous system, have been critically reviwed (Mason *et al*., 1990; Whitacre, 2001; Arnaud, 2009; Zandman-Goddard *et al*., 2012; Zhang *et al*., 2012). The experimental models used for this research included adrenalectomy, gonadectomy or delivery of exogenous hormones (Bonthuis *et al*., 2010).

While studying the role of Nitric Oxide (NO) in the development of The Experimental Autoimmune Encephalomyelitis (EAE) in the DA, Lew, PVG and BN rat (Willenborg *et al*., 1999; 2007; Staykova *et al*., 2008), it became clear that about 30 % of the male PVG rats develop not only EAE but also Adjuvant Arthritis (AA). (In 18 years of experimental work, in only seven male Lewis rats, did I observe swelling of the ankles after active induction of EAE).

The aim of this brief report is to point to the naturally occurring gonadal steroids' disbalance in a high percentage of the male PVG rats, which could be used in studies on poly-autoimmunity as well as in studies on sexual differentiation of brain and behavior.

2. MATERIALS AND METHODS

Male PVG rats, 10-20 week old, were bled via the lateral tail vein between 12 and 12.30 noon and immunized in both hind footpads with a total of 200 μL emulsion containing 30 μg guinea pig myelin basic protein and complete Freund's adjuvant (CFA, 4 mg ml^{-1} *Mycobacterium butyricum* in IFA, Difco Laboratories, Detroit, USA). The progesterone and testosterone serum levels were tested with the routine methods in ACT Pathology, Canberra. Based on the testosterone levels, two groups were formed: one with normal values (control, n = 4) and one with levels below the sensitivity of the method (0.69 nM L^{-1}) (experimental, n = 6). EAE clinical scores were assigned according to the accepted scale from no signs (score 0) to difficulty righting (score 3) (Staykova *et al*., 2002). Around day 13 after the immunisation, the animals in the control group started showing the typical neurologic signs of EAE that progressed from flaccid distal part of the tail (score 1) to entire tale flaccid (score 2). In the experimental group the paws and the ankles of all six rats started swelling eight-ten days after the immunisation and made difficult the evaluation of EAE. Swelling of hindpaws was quantified by measuring the thickness of the ankle from medial to lateral malleous with a constant tension caliper. On day 15 all animals were euthanized and the lumbar spinal cords and ankles were fixed in formalin. After decalcification, the joints were processed by the routine paraffin method. Paraffin

sections from both types of specimens (spinal cord and joints) were stained with haematoxylin-eosin. All animals received care that met the standards of the Animal Ethics Committee of the Australian National Health and Medical Research Council.

3. RESULTS AND DISCUSSION

The histopathologic picture of the lumbar spinal cords taken from male PVG rats, with normal testosterone levels or with testosterone levels below the sensitivity of the test (**Fig. 1 table**), was the same-with meningeal mononuclear cell inflammation (**Fig. 1A and C**). Thus, both groups developed comparable EAE.

In contrast, the mean ankle thickness increased from 6.8±0.2 mm (before immunization) to 12.4±0.9 mm (on day 15) in the group with low testosterone levels and there was a clear difference in the histology of the joints - normal for the control group and arthritic for the experimental group (**Fig. 1B and D**). This seems not to be dependent on the age of the animals in the experimental group although the numbers are too small for conclusions. In all AA rats there was a strong involvement of polymorph nuclear cells (**Fig. 1E**) that is typical for inflammatory cartilage destruction in rat adjuvant arthritis and was also reported for patients with rheumatoid arthritis (Glenn *et al*., 1977; Mohr *et al*., 1981; Eden *et al*., 2001; Ribbhammar *et al*., 2003; Hutsona and Hasthorpe, 2005).

Different rat strains have different sensitivity to T cell mediated autoimmunity (Swanborg, 2001). In EAE (actively induced with myelin basic protein in CFA) the order for the males is DA = Lew > PVG > BN (Willenborg *et al*., 2007). With respect to AA, the male DA rat is also most susceptible and the condition could be induced even with incomplete Freund's adjuvant (Mason *et al*., 1990; Ribbhammar *et al*., 2003). In the studies on AA in male Lew rat were used complete Freund's adjuvants with either *Mycobacterium tuberculosis* (Kong *et al*., 1999) or *M. butyricum* (Billiau and Matthys, 2001).

Progesterone (nM/L)	Testosterone (nM/L)	Ratio
8.7	9.7	0.9
12.6	3.1	4.1
12.9	13.3	0.97
22.1	18.7	1.2
14.1 ± 6.7	11.2 ± 6.5	
14.3	<0.69	>21
15.1	<0.69	>22
15.3	<0.69	>22
18.4	<0.69	>27
28.5	<0.69	>41
31.8	<0.69	>46
20.6 ± 7.6	<0.69*	

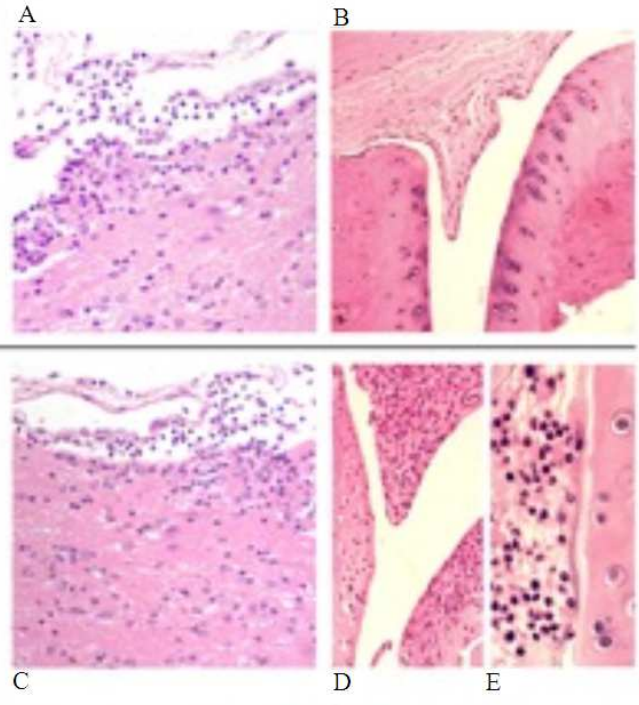

Fig. 1. Male PVG rat levels for progesterone and testosterone and histology of spinal cords and joints. Table: individual values of gonadal hormones in the control and experimental group. The pictures for haematoxylin-eosin stained lumbar spinal cord (A = control group; C = experimental group) and ankle joint (B = control group; D and E = experimental group) are representative for the individual animals in each of the two groups. Original magnification ×40 (A, B, C, D) and ×100 (E)

4. CONCLUSION

When the topic is poly-autoimmunity or multiple autoimmune syndrome, i.e., co-existence of more than two autoimmune diseases, than genetic analysis of the affected families helps to assess the risk (Ribbhammar *et al*., 2003; Criswell *et al*., 2005; Perez-Fernandez *et al*., 2012). On the other hand, models that reproduce autoimmune conditions (or phases of these conditions) are needed for experimental research.

Here we provide one more proof for the role of the endocrine system as a contributory cause in determining the susceptibility to two autoimmune conditions-EAE and AA. The fact that a good percentage of the male PVG rats have the androgen insensitive syndrome (previously called "testicular feminization syndrome", Hutsona and Hasthorpe, 2005) can be used in studies on poly-autoimmunity as well as in studies on variations in the sexual differentiation of brain and behavior (Bonthuis *et al*., 2010; Zuloaga *et al*., 2011).

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