Apomorphine-Susceptible and Apomorphine-Unsusceptible Wistar Rats Differ in Their Susceptibility to Inflammatory and Infectious Diseases: A Study on Rats with Group-Specific Differences in Structure and Reactivity of Hypothalamic–Pituitary–Adrenal Axis

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Variability in susceptibility to diseases is a well known phenomenon that has been attributed to genetic and environmental factors. At the level of the immune system, the reactivity of two types of T helper cells (Th1 and Th2 cells) plays an important role in determining disease susceptibility. Inflammatory (autoimmune) diseases are stimulated by cytokines produced by Th1 cells. Th2 cytokines stimulate antibody production (e.g., IgE) and eosinophilia as observed in allergic reactions or during parasitic infections. We describe here that the reactivity in a Th1 or a Th2 disease model significantly differs between individual rats that show group-specific differences in reactivity of the hypothalamic–pituitary–adrenal (HPA) axis, as well as in their behavioral responses to stress.

We used two outbred lines of Wistar rats, apomorphine-susceptible rats that have a relatively hyperreactive HPA axis (APO-SUS) and apomorphine-unsusceptible rats that have a relatively hyporeactive HPA axis (APO-UNSUS). APO-SUS, but not APO-UNSUS, rats generated a vigorous, Th2-dependent IgE response after infection with the nematode *Trichinella spiralis*. In contrast, APO-UNSUS, but not APO-SUS, rats were susceptible for Th1-mediated experimental autoimmune encephalomyelitis. Investigation of cytokine responses of splenocytes revealed that the ratio of mRNA expression for Th1-derived interferon (IFN)-γ and mRNA expression of Th2-derived interleukin-4 (IL-4) was significantly smaller in APO-SUS than in APO-UNSUS rats.

In conclusion, individual differences in structure and reactivity of the neuroendocrine system co-occur with group-specific differences in susceptibility to inflammatory and infectious diseases.

Key words: experimental autoimmune encephalomyelitis; T cells; hypothalamus–pituitary–adrenal axis; rats; Trichinella spiralis; interferon-γ; interleukin-4

Individual differences in susceptibility to inflammatory and infectious diseases are thought to be determined by the interplay between genetic and environmental factors. The immune system plays a major role in the pathogenesis of inflammatory and infectious diseases. The neuroendocrine system and the immune system interact (Heijnen et al., 1990; Munck and Guyre, 1990; Madden et al., 1995). Thus, it is conceivable that the reactivity of the neuroendocrine system contributes to disease susceptibility.

We focused on two types of rats that are present in each unselected, outbred population of Wistar rats, namely “high responders to novelty” and “low responders to novelty” (Piazza et al., 1989, 1990a,b; Cools et al., 1990, 1993). Since 1985, Cools et al. have been able to breed these two types of individuals. They have shown that the bimodal variation in apomorphine susceptibility, the original selection criterion for the breeding, is consistently coupled to a bimodal variation in a great variety of neuroanatomical, neurochemical, endocrinological, and behavioral features. Rats marked by a high apomorphine susceptibility (APO-SUS) are high responders to novelty in terms of behavioral response (high exploratory activity) and endocrinological responses (high and long-lasting plasma release of ACTH and corticosteroids). Rats marked by low apomorphine susceptibility (APO-UNSUS) are low responders to novelty in terms of behavioral response (low exploratory activity) and endocrinological responses (low and short-lasting release of ACTH and corticosteroids; Cools et al., 1990; Rots et al., 1995, 1996a,b).

At the level of the immune system, it is thought that two types of T helper cells play an important role in determining susceptibility to disease. Th1 cells predominantly produce γ-interferon (IFN-γ) and interleukin-2 (IL-2), which promote cellular immunity (Mosmann and Sad, 1996). In experimental models of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE), the Th1 type effector cell response is dominant (Mosmann and Sad, 1996). Th2 cells secrete IL-4, IL-5, and IL-10, which provides help for B cell differentiation and humoral immune responses (Mosmann and Sad, 1996). The immune response to infection with parasitic helminths such as *T. spiralis* involve elevated IgE antibody production, eosinophilia, and mastocytosis (Finkelman et al., 1991). These responses are all stimulated by Th2-derived cytokines (Finkelman et al., 1991; Mosmann...
produce the Th1 cytokine IFN-γ after mitogenic stimulation, splenocytes (10^6/ml) of APO-SUS and APO-UNSUS rats were cultured in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with antibiotics and 5% heat-inactivated FCS (Gibco) with the polyclonal activator PMA (10 ng/ml) plus ionomycin (400 ng/ml) for 20 hr. Supernatants were harvested, and the concentration of IFN-γ was determined by ELISA (Van der Meide et al., 1990).

Because quantitative measurement of serum level of IL-4 are not yet available, the expression of IL-4 mRNA as well as of IFN-γ mRNA was determined by quantitative RT-PCR to gain insight into the relative contribution of Th1 or Th2 type responses in APO-SUS and APO-UNSUS rats. At the time point when the above-mentioned supernatants were collected, cells were harvested and RNA was extracted by the use of RNAzol B (Campro Scientific, Veenendaal, The Netherlands). Two micrograms of RNA were reverse transcribed into cDNA using AMV reverse transcriptase and oligo-dT 12–18 oligonucleotide as primer according to the manufacturer’s protocol. Quantitative competition PCR was performed as described by Siegl et al. (1993, w 4°C), freely provided us with a competitor plasmid containing primers for β-actin, IFN-γ, and IL-4.

Serial dilutions of competitor fragment were coamplified with fixed amounts of cDNA. The PCR product for the sample cDNA and the competitor differ in size so that the relative intensities of the two products can be compared. For calculations, 1 unit of cDNA signal was defined as the amount that resulted in equal density of competitor and target cDNA at 1 μl of 1:50 dilution of the competitor. The following primer pairs were used: β-actin sense, 5′-CTATCGCAATGACCGGTTC; antisense, 5′-CTTAGGATGGGGTGTTCGCT; IFN-γ sense, 5′-CCTCTGGCTGTACTG; antisense, 5′-CTCCTTGGCTGTACCG; IL-4 sense, 5′-CTCGACGATGTTGTGCC; antisense, 5′-TCTAGATGGTTTCCCAGGA. These primer pairs gave rise to PCR products for the β-actin gene of 762 bp for sample cDNA and 601 bp for competitor fragment. IFN-γ sample cDNA yields a fragment of 419 bp, whereas the competitor results in a fragment of 319 bp. For IL-4, sample cDNA results in 275 bp and competitor cDNA in 178 bp. cDNA was amplified in a 20 μl reaction volume containing 2 μl of 10X PCR buffer, 0.25 mM each dNTP, 50 ng/ml of the appropriate primer pair, 2 mM MgCl2, and serial dilutions of competitor fragment. After 5 min denaturation at 94°C, cDNA samples were subjected to cycles of denaturation (15 sec at 94°C), annealing (15 sec at 60°C), and extension (15 sec at 72°C) using the thermal cycler 9600 (Perkin-Elmer). To correct for variations among different preparations, cDNA samples were adjusted to equal input cDNA concentrations based on their β-actin content before determination of cytokine cDNA content. Control PCRs without cDNA were performed in all experiments to exclude contamination.

Data analysis. Data were analyzed by Mann–Whitney U test or Fisher’s exact test, and p < 0.05 was considered statistically significant.

RESULTS

Susceptibility to experimental autoimmune encephalomyelitis

APO-SUS and APO-UNSUS rats showed a clear difference in susceptibility to EAE (Fig. 1 and Table 1). APO-SUS rats were less susceptible for EAE. The incidence of disease and the mean cumulative clinical score were lower in APO-SUS animals than in APO-UNSUS animals. In addition, there was a significant difference in the kinetics of disease development. APO-SUS animals showed a significant delay with respect to onset of the disease when compared with APO-UNSUS rats. The first symptoms of disease were observed only at day 12 after inoculation in APO-SUS rats. In contrast, APO-UNSUS rats showed the first symptoms of the disease at day 8 after inoculation, the degree of paralysis increased reaching maximal levels at day 11 after inoculation in these animals; subsequently, disease activity gradually decreased, and complete remission was observed after 18 d. There was no group-specific difference in duration of the disease.

Response to inoculation with Trichinella spiralis

APO-SUS rats developed a higher level of anti-T. spiralis IgE than APO-UNSUS rats (Fig. 2). In eight of nine APO-SUS animals, T. spiralis-specific antibodies of the IgE subclass could be detected,
whereas only two of seven APO-UNSUS rats developed detectable levels of parasite-specific IgE.

There were no group-specific differences in the levels of IgG or IgA specific for *T. spiralis* (Table 2).

**Production and/or mRNA expression of Th1 and Th2 type cytokines**

The splenocytes of APO-SUS and APO-UNSUS rats did not show group-specific differences in the capacity to produce IFN-γ after mitogenic stimulation (Table 3). Moreover, neither the expression of IFN-γ mRNA nor that of IL-4 mRNA differed between both lines, although the expression of IL-4 was slightly, but not significantly, greater in APO-SUS rats than in APO-UNSUS rats (*p* = 0.06; Table 4). However, the relative contribution of Th1 cells versus Th2 cells, as expressed in terms of the ratio IFN mRNA/IL-4, was significantly greater in APO-UNSUS rats than in APO-SUS rats (*p* = 0.03; Table 4).

**DISCUSSION**

The present study confirms and expands our previous findings that the susceptibility to EAE was significantly smaller in APO-SUS rats than in APO-UNSUS rats; when compared with APO-UNSUS rats, the severity of clinical symptoms of EAE was significantly less and the onset was significantly delayed (see Fig. 1 and Table 1 in Cools et al., 1993). In contrast, APO-SUS rats have a significantly larger response to infection with the nematode *T. spiralis* than APO-UNSUS rats; the level of parasite-specific IgE was significantly higher in APO-SUS rats than in APO-UNSUS rats, although the levels of parasite-specific levels of IgA and IgG did not differ between both lines in this model for Th2-dependent infectious diseases (Table 2, Fig. 2). In line with these data, the
The present study shows that the relative contribution of Th1 and Th2 type responses significantly differed between both lines; as shown in Table 4, the ratio of the mRNA expression for the Th1 cytokine IFN-γ and for the Th2 cytokine IL-4 in splenocytes was much smaller in APO-SUS rats than in APO-UNSUS rats. These data together show that APO-SUS and APO-UNSUS rats show group-specific differences in their susceptibility to inflammatory and infectious diseases, respectively.

Table 4. Expression of mRNA for IL-4 and IFN-γ after polyclonal activation of splenocytes from APO-SUS and APO-UNSUS rats

<table>
<thead>
<tr>
<th></th>
<th>APO-SUS (n = 6)</th>
<th>APO-UNSUS (n = 6)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4*</td>
<td>0.5 (0.14)</td>
<td>0.14 (0.04)</td>
<td>0.06</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1.7 (0.2)</td>
<td>1.17 (0.17)</td>
<td>NS*</td>
</tr>
<tr>
<td>Ratio IFN-γ/IL-4</td>
<td>3.5 (0.5)</td>
<td>6.4 (0.75)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Splenocytes from naive APO-SUS and APO-UNSUS rats were cultured for 20 hr in the presence of PMA and ionomycin. Cells were harvested and RNA was extracted. The level of expression of mRNA for IL-4 and IFN-γ was determined by quantitative competition RT-PCR.

a Mann–Whitney two sample test.
*b The level of IL-4 and IFN-γ cDNA is expressed in units as defined in Materials and Methods.
NS, not significant.

The role of HPA axis reactivity in the inflammatory process in the disease EAE has been suggested in other animal models as well. In the inbred strain of Lewis rats, which are highly susceptible to EAE, it has been shown that the responsiveness of the HPA axis is impaired (Sternberg et al., 1989a, b). The impaired functioning of the HPA axis in Lewis rats has been ascribed to a defect in the hypothalamic secretion of CRH (Sternberg et al., 1989b). In this respect it is of interest that APO-UNSUS animals express lower levels of CRH mRNA in the paraventricular nucleus of the hypothalamus than APO-SUS animals (Rots et al., 1995). Administration of glucocorticoids to inflammatory autoimmune disease-sensitive Lewis rats renders them into resistant animals (Sternberg et al., 1989a, b). On the other hand, resistant Fischer F344 rats can be rendered into highly susceptible animals by administration of the glucocorticoid receptor antagonist RU 486 (Sternberg et al., 1989a, b). In the mouse model, two inbred strains have been described that differ in the dominance of responses of Th1 or Th2 cells as well as in the reactivity of the neuroendocrine system. BALB/c mice respond predominantly with a Th2 type response, whereas C57/b16 mice respond with a Th1 type response (Scott et al., 1989; Heinzel et al., 1991). It is of interest that these two strains display differences in the reactivity of the HPA axis that are similar to the differences between APO-SUS and APO-UNSUS rats: stress-induced increases in IL-4 in BALB/c mice are larger than in C57/b16 mice (Shanks et al., 1994). Together, these data support the hypothesis that the structure and, especially, the reactivity of the HPA axis direct the Th1/Th2 balance.

In comparison with the above-mentioned animal models, the model of APO-SUS and APO-UNSUS rats has several advantages, of which only two are mentioned below. First, the procedure used to breed APO-SUS and APO-UNSUS rats guarantees the maintenance of the originally present genotypic heterogeneity, apart from the alleles at the loci involved in the determination of the selected traits; this matches the human situation far better than animal models with different inbred strains of rodents because such inbred strains, unlike humans, are each marked by a genotypic uniformity. Second, the available knowledge about group-specific differences in structure and function of the HPA axis in Lewis rats has been ascribed to a defect in the hypothalamic secretion of CRH (Sternberg et al., 1989b). In this respect it is relevant to mention that the group-specific differences in the structure and reactivity of the brain of APO-SUS and APO-UNSUS rats are consistently and causally coupled to group-specific vulnerability for immune diseases and different coping styles.

Recently, we have found that APO-SUS and APO-UNSUS rats differ also in the adrenergic reactivity of the peripheral and CNS. Apart from the finding that the basal plasma level of adrenaline is lower in APO-SUS rats than in APO-UNSUS rats that may result in relatively hypersensitive β2-adrenergic receptors, the stress-induced increase in adrenaline is much higher in APO-SUS rats than in APO-UNSUS rats (Rots, 1995). Therefore, the adrenergic system may be more effective in modulating responses in APO-SUS rats than in APO-UNSUS rats. Cells of the immune system express β2-adrenergic receptors and from in vitro experiments it is known that adrenaline can selectively influence the reactivity of Th1 or Th2 cells (Johnson and Gordon, 1981). The increase in intracellular cAMP after activation of β2-adrenergic receptors...
results in increased IL-4 production (Paul-Eugene et al., 1993; Lacour et al., 1994; Katamura et al., 1995). Moreover, β2-adrenergic agonists can stimulate IL-4-dependent IgE synthesis (Paul-Eugene et al., 1993, 1995). We have data showing that β2-adrenergic agonist inhibit IFN-γ production, resulting in a shift toward Th2 type responses (A. Kavelaars, unpublished data).

Thus, the relatively increased responsiveness of Th1 type T cells.

REFERENCES


