



High plasma IgE levels within the Scandinavian wolf population, and its implications for mammalian IgE homeostasis

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Abstract

Immunoglobulin E (IgE) serves as an important link between innate and adaptive immunity through its ability to bind high affinity receptors on mast cells and basophils. Large differences in IgE levels may here affect this important link, and IgE levels in natural non-domestic animal populations may therefore be very informative concerning the levels of IgE that this system have been balanced against during recent mammalian evolution. However, very few such studies have been performed. Here, we present an analysis of total IgE levels in 65 Scandinavian wolves: 57 free living (wild), and 8 wolves in captivity (Zoo). The 57 wild wolves correspond to approximately 30% of the entire wolf population in Sweden and Norway and thus represent a large fraction of the entire population, making this a unique sample from a wild canine population. The median IgE level in these wolves was 67 $\mu\text{g/ml}$, which is approximately twice the level seen in domestic dogs and more than 100 times the levels in non-atopic humans. The collected information from domestic and wild populations now indicate that the very low IgE levels observed in man and laboratory rodents are most likely an effect of a life in a relatively parasite free environment, and that total IgE levels under maximally stimulatory (normal) conditions may reach 100–200 $\mu\text{g/ml}$.

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1. Introduction

All placental mammals in which a careful analysis of both genomic sequences and serum immunoglobulins have been performed express five immunoglobulin classes: IgM, IgD, IgG, IgE and IgA. The only exception is rabbits, which appear to lack a gene for IgD (Lanning et al., 2003). Of these five Ig classes, IgG and IgE were the last two to evolve as separate immunoglobulin classes. The gene duplication give rise to these two Ig classes seems to have occurred during early mammalian evolution, 210–350 million years ago (van Rheede et al., 2006; Vernersson et al., 2002, 2004; Woodburne et al., 2003). Interestingly, following this gene duplication, IgG has become the Ig class that is present in the highest concentrations. IgG levels between 8–16 mg/ml is normally seen in human plasma. In con-

trast, IgE is present in very low concentrations, in non-atopic humans levels in the range of 1–400 ng/ml. The difference in concentration is thereby 10,000 to 1 million times.

Similar to humans, most laboratory strains of rats and mice have very low levels of plasma IgE, below 400 ng/ml. IgE levels in the range of 1 ng to a few hundred ng have therefore been considered as the standard for domestic animal populations. Until recently, almost no reliable studies of other animal species than laboratory rodents and humans have been available that could challenge this view of IgE. However, a few years ago reliable tests for IgE levels in both dog and horse were developed, and the IgE levels were found to be very high compared to the levels observed in mice, rats and humans. In 76 adult dogs, the IgE levels were found to range between 1 and 41 $\mu\text{g/ml}$ serum, which is almost 100 times the levels seen in non-atopic humans (Ledin et al., 2006). Similarly, in 26 Islandic horses, the levels were in the range of 30–180 $\mu\text{g/ml}$ (Wagner et al., 2003).

To obtain a better view of what factors that are of importance in determining total IgE levels, and how high the IgE levels can become under maximally stimulatory conditions studies of

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Table 1
Sex, age, weight, location of capture and total IgE levels in 57 free-living Scandinavian wolves

Wolf	Sex	Age and weight	IgE/ml	Age and weight	IgE/ml	Age and weight	IgE/ml	Age and weight	IgE/ml	Location
1	F	0 (35 kg)	51							Filipstad
2	M	7–8 (53 kg)	35							Hagfors
3	F	5–6 (38 kg)	47							Hagfors
4	M	0 (38 kg)	89	5 (45 kg)	102					Leksand-Grangräde
5	F	4–5 (35 kg)	44	7–8 (41 kg)	12					Leksand
6	M	0 (37 kg)	61							Hagfors
7	M	0 (37 kg)	22							Hagfors-Telemark
8	M	0 (35 kg)	33	1 (48 kg)	63					Hagfors
9	M	5–6 (53 kg)	63							Årjäng-Kongsvinger
10	F	5–6 (40 kg)	48	8–9 (44 kg)	19					Årjäng-Kongsvinger
11	F	0 (36 kg)	68							Årjäng-Kongsvinger
12	F	4–5 (42 kg)	21							Grangärde
13	M	2–3 (53 kg)	42							Leksand-Hedmark
14	M	2 (49 kg)	71							Leksand-Tyngsjö
15	M	3 (51 kg)	66	5 (51 kg)	48	5 (47 kg)	31	7 (48 kg)	2	Nyskoga
16	F	4 (42 kg)	62	6 (37 kg)	20					Nyskoga
17	M	5–7 (49 kg)	48	8–9 (48 kg)	6	9–10 (50 kg)	0			Bograngen
18	M	0 (40 kg)	42							Bograngen
19	F	0 (31 kg)	81							Grangärde
20	M	0 (38 kg)	69							Grangärde
21	F	0 (33 kg)	61							Hasselfors
22	F	4–5 (38 kg)	57							Hasselfors
23	M	3–4 (49 kg)	72	5–6 (47 kg)	51					Hasselfors
24	F	0 (24 kg)	50							Hasselfors
25	F	0 (33 kg)	34							Nyskoga
26	M	0 (36 kg)	51							Nyskoga
27	M	4–5 (52 kg)	26	5–6 (51 kg)	23	6–7 (51 kg)	14	7–8 (49 kg)	11	Gråfjell
28	F	3–4 (43 kg)	66	4–5 (41 kg)	25	5–6 (41 kg)	103			Gråfjell
29	F	1 (39 kg)	73							Langedrag
30	F	4 (39 kg)	11							Tyngsjö
31	M	0 (39 kg)	27							Nyskoga
32	F	0 (30 kg)	64	1 (38 kg)	78	2 (41 kg)	47			Nyskoga
33	F	0 (38 kg)	68	1 (38 kg)	87					Nyskoga
34	M	1–2 (45 kg)	92	3 (51 kg)	81					Dals-Ed - Halden
35	F	0 (34 kg)	49	1 (39 kg)	39	2 (39 kg)	81			Årjäng-Kongsvinger
36	F	0 (30 kg)	48							Galskogen
37	F	0 (31 kg)	68	1 (35 kg)	54	2 (35 kg)	58			Årjäng-Kongsvinger
38	F	4–5 (46 kg)	60							Glaskogen
39	M	2 (52 kg)	52							Glaskogen
40	M	1 (46 kg)	53	2 (44 kg)	75	3 (42 kg)	72			Glaskogen
41	M	1–2 (47 kg)	58							Norrbotten
42	M	3–4 (51 kg)	61	4–5 (45 kg)	72					Leksand
43	M	0 (35 kg)	49							Nyskoga
44	F	3–4 (36 kg)	15							Grangärde
45	F	4 (46 kg)	95							Ddl-Ed
46	F	0 (31 kg)	22							Gråfjell
47	F	0 (30 kg)	48							Gråfjell
48	M	1 (45 kg)	10 (dead)							Jämtland
49	M	1 (38 kg)	40							Nora-Sandra
50	F	0 (32 kg)	55							Nyskoga
51	M	2 (44 kg)	92	3 (48 kg)	75					Djurskog
52	F	1 (35 kg)	74							Koppang
53	M	0 (36 kg)	37							Gråfjell
54	F	1 (42 kg)	37							Skoga
55	M	2 (51 kg)	74							Stöllet
56	M	2 (47 kg)	33							Koppang
57	M	1 (47 kg)	103							Skoga

All IgE levels are in µg/ml.

wild (non-domestic) populations with an expected heavy parasite load may be very informative (Hellman, 2007). This is also a very interesting question from an evolutionary perspective as IgE is the link between mast cells and adaptive immunity.

A more detailed picture of the physiological levels of IgE, under normal wild condition, is therefore of major importance for our understanding of the conditions the IgE mast cell system has been adapted to during the past 200 million years of mammalian evolution. We have therefore here extended the analysis of IgE levels into a wild canid population the gray wolf (*Canis lupus*).

2. Materials and methods

2.1. Animals and collection of serum samples

Fifty-seven free-ranging Scandinavian gray wolves (*C. lupus*) were included in this study. The wolves were chemically immobilized from a helicopter in winter (December–March) from 1998 through 2004 in Sweden and Norway (59–61°N, 11–15°E) for radio collaring, health assessment, and sampling of biological materials (Arnemo and Fahlman, 2007). Eighteen animals were immobilized two to four times in order to change the radio collar (Table 1). All recaptures occurred in different seasons (9–38 months apart).

Blood was collected from the cephalic vein using 10 ml evacuated tubes with gel and clot activating factor (Venoject® II system, Terumo Europe N.V., Leuven, Belgium). The tubes were kept at room temperature for 1–2 h to ensure complete coagulation. Serum was then separated by centrifugation at $1500 \times g$ for 10 min and stored in 2 ml cryogenic vials (Nalgene®, Nalge Company, Rochester, NY, USA) at -20°C for 0–5 years. Serology (ELISA and Western Blot) for sarcoptic mange was carried out at the National Veterinary Institute, Uppsala, Sweden.

Capture of free-ranging wolves was approved by the Ethical Committee on Animal Experiments in Umeå, Sweden and by the National Animal Research Authority in Oslo, Norway.

2.2. ELISA measurements of total IgE levels

Total IgE levels were determined by ELISA as described in Ledin et al. (2006).

3. Results

3.1. Animals and serum sampling

Serum or plasma samples from 57 free-living Scandinavian wolves were collected from wolves in Norway and Sweden during more than 6 years of intensive field research (1998–2004). The wolves were chemically immobilized from a helicopter, and they were primarily from the central region of Sweden and Norway, and one from the northern part of Sweden. These 57 wolves correspond to approximately 30% of the entire wolf population in Sweden and Norway, and thus represent a large fraction of the entire population and a unique sample from a wild canine population. The regions of capture for sampling are indicated

Table 2

Total IgE levels in eight wolves living in a Norwegian Zoo

Wolf	Sex	IgE/ml	Location
58	M	71	Zoo (Langedrag)
59	F	98	Zoo (Langedrag)
60	F	85	Zoo (Langedrag)
61	Puppy	138	Zoo (Langedrag)
62	Puppy	124	Zoo (Langedrag)
63	Puppy	131	Zoo (Langedrag)
64	Puppy-	84	Zoo (Langedrag)
65	Puppy	95	Zoo (Langedrag)

All IgE levels are in $\mu\text{g/ml}$.

in Table 1. Most of the wolves were sampled only once, however for some two, three or even four samples were collected separated in time over several years. Serum or plasma samples from eight wolves in captivity are also included in this study.

3.2. Analysis of total serum IgE levels

Using a recently developed ELISA assay for canine IgE, these sera and plasma samples were analyzed for their total IgE levels (Ledin et al., 2006). The samples were analyzed several times and with several dilutions to ensure accurate values. A summary of the data of the free-living 57 wolves is found in Table 1, and the values of the 8 wolves in captivity are found in Table 2. The median value of all the 94 samples was $67 \mu\text{g/ml}$, which is almost twice the value previously observed in a panel of 76 adult domestic dogs (Ledin et al., 2006). The median value of the free-living wolves was $54 \mu\text{g/ml}$, whereas the median value of the IgE levels in wolves in captivity was almost twice as high $103 \mu\text{g/ml}$. The reason for this high value may lay in the fact that the wolves in captivity stay in the same area for extended periods, and the risk of re-infection with intestinal parasites may thereby increase substantially.

3.3. Skin parasites and their effect on IgE levels

The wild wolves were also examined for signs of scabies (caused by *Sarcoptes scabiei*) and their sera were analyzed for scabies-specific antibodies by ELISA. Wolves number 2, 9, 13, 22, 23 (both samples), 29, 34 (second sample), and 40 (second and third sample) were found to be ELISA positive, whereas wolves number 9, 13, 22, 23 (first sampling), 34 (second sampling), and 40 (second and third sampling) also were clinically positive. The mean and median serum IgE levels for these samples were 62 and $68 \mu\text{g/ml}$, respectively, whereas the median value of all the 94 wolf samples was $67 \mu\text{g/ml}$ as described above. Judging from these results, scabies does not seem to have a major impact on IgE levels in wolves.

3.4. Effects of age on IgE levels

A large variation in IgE levels between animals and also in the same animal over time was seen within this wolf population. A tendency of decreasing IgE levels with age was also observed.

However, wolves of less than 1 year in age did not have IgE levels that markedly differed from adult wolves of 1–5 years in age.

4. Discussion

Studies of IgE and IgE regulation has until recently been performed almost entirely in human and domestic rodents, and then primarily on parasite free populations. The results from studies of IgE levels in dogs and horses, which frequently are parasite infested have recently shown that their IgE levels exceeded previously obtained levels in man and laboratory rodents by almost 100 times (Ledin et al., 2006; Wagner et al., 2003). This large difference probably results from differences in parasitic load (Meeusen, 1999). It is difficult to keep horses free of parasites since they frequently are kept on open pastures together with other horses, and thereby may be rapidly re-infected after treatment with anti-parasitic drugs. Similarly dogs may also become re-infected relatively rapidly after parasite treatment due to their daily outdoor activities. However, studies of intestinal helminth infections in domestic dogs in the Nordic countries have shown that a relatively low percentage of these dogs actually are infected (3.5–7%) (Pullola et al., 2006), and a high percentage of the dogs are being treated with anti-parasitic drugs at least once a year. The high levels of IgE observed in dogs may therefore indicate that these levels stay high for long periods after removal of the parasites and only slowly adjust to new conditions (Ledin et al., 2006). This interpretation is supported by at least two studies of humans where high levels of IgE have persisted for several years after the patients have been treated for their parasite infections (Iancovici Kidon et al., 2005; Mitre and Nutman, 2006).

Analysis of Wistar rats infected by *Angiostrongylus costaricensis*, an intestinal helminthic worm parasite, during 50 days post infection showed that IgE levels increased from below 2 µg/ml to almost 100 µg/ml after 25 days of infection and stayed above 50 µg for an additional 25 days to the end of the study (Serra et al., 2003). As already mentioned, IgE levels in heavily parasite infested humans are also quite high (Iancovici Kidon et al., 2005; Scaglia et al., 1979). However, levels in the range of what is observed in dogs, wolves and horses (and some studies of rodents) are rarely seen (Iancovici Kidon et al., 2005; Scaglia et al., 1979). This raises the question as to what levels IgE may reach in non-domestic animal populations. A recent study of wild rats and mice showed IgE levels much higher than in their domestic conspecifics, with the mice having levels around 1 µg and rats around 10–15 µg /ml, which correspond to a 12-fold difference in mice and a 4-fold difference in rats (Devalapalli et al., 2006). Interestingly, four wild mice with four distinct backgrounds that were kept in laboratory environment had similar or even lower levels of IgE than the laboratory mice, which indicate that environmental rather than genetic factors are the major contributors to differences in IgE levels within species.

In the present study, a large variation in IgE levels between animals and also in the same animal over time was observed.

This is frequently seen also in other species where IgE levels in the same animal may change several fold or even an order of magnitude over only a few weeks time (Ledin, and Hellman unpublished observations). IgE is apparently under a very dynamic and complex control, and small changes can lead to both dramatic and rapid changes in IgE levels [Hellman, 2007].

Interestingly, wolves of less than 1 year in age did not have IgE levels that markedly differed from adult wolves of 1–5 years in age. This in sharp contrast to previous studies in several species including dogs and horse. In both dogs and horses, IgE levels in very young animals are very low, sometimes almost undetectable (Ledin et al., 2006; Wagner et al., 2003). In the wolves, we actually found the highest values of all individuals in three very young animals. Three of the puppies in captivity had values of 138, 124 and 131 µg/ml of IgE (Table 2). The reason for this is not fully understood but may be due to very early infection by intestinal helminthes, which in turn induces a rapid and strong IgE response. We also observed a tendency of decreasing IgE levels with age. The reason for this is also not fully understood but may be due to a successful immunity to the parasites and thereby decreased or fully cleared infection.

Concerning parasites in the wolf population, no major impact was observed of scabies on IgE levels, which clearly indicates that there are major differences in IgE inducing effects of skin and intestinal parasites.

When looking at the total IgE levels in our wolf population, we observed that the levels were higher than earlier found in domestic dogs. The difference was clear but not as dramatic as we had expected—only a two-fold higher level of IgE in the wild canids. The relatively small difference observed between IgE levels in dogs and wolves indicates that the levels observed in wolves approach the maximal levels that can be obtained even after activation of all the regulatory pathways affecting IgE homeostasis (Hellman, 2007). The results referred to here from wolves, dogs, horses and some studies in rodents now also indicate that the maximal levels are in the range of 100–200 µg/ml. Substantial differences may however result from genetic factors within, and between species.

In conclusion, we can say that one of the most important inducers of IgE production most likely is parasite infections and in particular helminthic parasites, and that IgE levels in parasite infested populations may reach 100–200 µg/ml.

The IgE–mast cell system has in placental apparently been balanced against IgE levels in the range of a few hundred µg. However, almost all of our knowledge concerning IgE and IgE regulation come from man and domestic rodents that generally have IgE levels far outside of this “balanced” range. Much of the dysregulation seen in man today with 20–30% of the population being allergic may be caused by the fact that the IgE mast cell system never has been balanced against these low IgE levels, in an evolutionary timescale. This information should be taken into consideration when trying to design new ways to battle IgE mediated (atopic) allergies, one of the major challenges of human medicine of the 21st century.

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