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European Journal of Pain

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## The passive transfer of immunoglobulin G serum antibodies from patients with longstanding Complex Regional Pain Syndrome

Andreas Goebel<sup>a,b,d,\*</sup>, Maria I. Leite<sup>b</sup>, Li Yang<sup>b</sup>, Robert Deacon<sup>c</sup>, Cruz M. Cendan<sup>d,e</sup>, Andrew Lewis<sup>c</sup>, Angela Vincent<sup>b</sup>

<sup>a</sup> Pain Research Institute, University of Liverpool, Liverpool, UK

<sup>b</sup> Weatherall Institute of Molecular Medicine, John Radcliffe Hospital, University of Oxford, Oxford, UK

<sup>c</sup> Experimental Psychology, University of Oxford, Oxford, UK

<sup>d</sup> Molecular Nociception Group, University College London, UK

<sup>e</sup> Department of Pharmacology and Institute of Neuroscience, Faculty of Medicine, University of Granada, Granada, Spain

### ARTICLE INFO

#### Article history:

Received 16 June 2010

Received in revised form 18 October 2010

Accepted 18 October 2010

Available online xxx

#### Keywords:

Complex Regional Pain Syndrome (CRPS)

Autoimmunity

Autoantibody

Chronic pain

Intravenous immunoglobulin (IVIG)

### ABSTRACT

**Background:** The aetiology of Complex Regional Pain Syndrome (CRPS) is unknown. Recent evidence suggests that there may be autoantibodies directed against peripheral nerves, but it is unclear whether such autoantibodies are merely biomarkers or whether they cause or contribute to the underlying pathology. The transfer of disease after injection of a patient's serum or IgG fraction into mice ('passive transfer') is the classic way to demonstrate a functional role of autoantibodies.

**Aims:** Based on previous preliminary results, we wished to investigate whether the transfer of IgG antibodies affected mouse behaviour or produced signs of CRPS.

**Methods:** We injected purified serum-IgG from 12 patients and 12 controls into groups of 6–10 mice (~17 mg/mouse intraperitoneally) on 2 consecutive days and looked for any evidence for altered behaviour or signs of CRPS. The observer, blinded as to test or control group, measured behaviour in the open field, stimulus-evoked pain and motor coordination, and inspected limbs for autonomic CRPS signs.

**Results:** Stimulus-evoked pain and autonomic signs were not detected, but CRPS-IgG induced significant depression of rearing behaviour (17.9 rears/3 min ( $n = 84$ ) vs. 22.1 rears/3 min ( $n = 83$ ),  $p = 0.0004$ ), confirming previous observations in a single case study. Moreover, motor impairment, one of the four cardinal signs of CRPS, was evident in the three CRPS-IgG injected groups tested with a sensitive rota-rod protocol ( $p < 0.0001$  vs. control-IgG injected groups).

**Conclusions:** These results lend support to a pathophysiological role for IgG autoantibodies in CRPS.

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*Cite this article as:* Andreas Goebel, Maria I. Leite, Li Yang, Robert Deacon, Cruz M. Cendan, Andrew Lewis, Angela Vincent, The passive transfer of immunoglobulin G serum antibodies from patients with longstanding Complex Regional Pain Syndrome. *Eur J Pain* xxx (2010) xxx–xxx [doi:10.1016/j.ejpain.2010.10.005]

Full text available online on ScienceDirect®, [www.sciencedirect.com](http://www.sciencedirect.com).

### 1. Introduction

Complex Regional Pain Syndrome (CRPS) is usually posttraumatic and restricted to one limb. Patients suffering from CRPS experience non-resolving pain, autonomic abnormalities, and limb dysfunction (Birklein, 2005). The aetiology of CRPS is unknown, but we have recently demonstrated a beneficial response to intrave-

nous immunoglobulins (IVIG) in a double blind crossover trial (Goebel et al., 2010), suggesting an immune contribution, and we and others have found that CRPS is associated with increased frequency of serum-autoantibody binding to rodent peripheral nerves (Blaes et al., 2004; Goebel et al., 2005b; Kohr et al., 2009). However, it is not yet clear whether these antibodies are causative or merely secondary biomarkers with no pathogenic role.

Passive transfer of disease to mice is the classic method for investigating a causative role for autoantibodies, as shown in many established auto-antibody-mediated conditions (Rose and Bona, 1993). Based on previous preliminary results in a single case study (Goebel et al., 2005a), we investigated whether the transfer of IgG

\* Corresponding author. Address: Pain Research Institute, Clinical Sciences Centre, University Hospital Aintree/The Walton Centre, Liverpool L9 7AL, England, UK. Fax: +44 151 529 5821.

E-mail address: [andreasgoebel@rocketmail.com](mailto:andreasgoebel@rocketmail.com) (A. Goebel).

antibodies affected mouse behaviour or produced signs of CRPS. We took advantage of IgG preparations obtained from pre-treatment sera taken from each of the 12 patients undergoing the recent successful IVIG trial (Goebel et al., 2010), and found significant effects on exploratory behaviour and motor function in injected mice compared with controls.

## 2. Methods

### 2.1. Patients, controls, and IgG preparation

Following ethics committee approval (UCLH research ethics, London, UK) and informed consent according to the Declaration of Helsinki, 150 ml of blood were taken, before treatment, from all patients ( $n = 13$ ) who had been enrolled into a randomized controlled crossover trial on IVIG treatment in CRPS. The patients' baseline characteristics are given in (Table 1). As controls, age and sex-matched healthy volunteers underwent a short interview, and gave 150 ml of blood if they had no significant pain problem, or known autoimmune disorders in first degree relatives. The blood samples were cold centrifuged at  $2500G \times 10$  min and serum was stored frozen at  $-80$  for later processing. Preparations from patients who completed the trial ( $n = 12$ ), and the matching volunteers, were purified in the week before each experiment. IgG fractions were prepared using a protein G column as previously described (Buckley and Vincent, 2005), and the concentrations were adjusted in Hartmann's solution to between 8 and 9 mg/ml. The samples were then anonymised and stored at  $4^\circ C$  until use.

### 2.2. Experimental design and behavioural tests

Female 6-week C57BL6 mice were acclimatized for 1 week, and either 6, or 10 (groups 2, 7 and 9) animals per group were weighed. Baseline tests for sensitivity to von Frey hairs were performed in some groups; the animals were then injected intraperitoneally (ip) on 2 consecutive days, observer blinded, with either patient- or matched control IgG preparations (1 ml/injection, 8–9 mg). Animals were assessed regularly for general appearance and behaviour in the cages. Paws were visually inspected for swelling and colour changes from day 2 to day 5 when the animals were sacrificed. Five hours after the second injection the mice were placed in a minimally anxiogenic open field for 3 min (Deacon et al., 2002). Rears (number of times the mouse stood completely erect on its hind legs), crossed squares, and the latency to the first rear were

recorded. In some groups tactile allodynia with von Frey hairs and heat hyperalgesia with a Hargreaves apparatus were recorded on day 3, and cold allodynia/hyperalgesia on a cold plate on day 4 (Abrahamsen et al., 2008). These methods are described in more detail in the supplementary material. All groups were tested for motor coordination on the rota-rod (Jones and Roberts, 1968) on day 4, either following a standard procedure described in the supplementary material (Zimmermann et al., 2007) or, in three pairs of control and CRPS-IgG injected mice, with a modified protocol. For this, the outcome was the rotational speed at fall, rather than the time on the rota-rod. If the mouse fell of the rota-rod within the first 10 s (speed 4 rotations/min) it was replaced, and the outcome was recorded as (speed at second fall + 4)/2; if a second fall occurred within the first 10 s, the outcome was recorded as (4 + 4)/2. The rotational speed was accelerated after 10 s at a rate of 20/min. This protocol corrects for the extra practice which the mouse receives during the failed runs, which is assumed would assist performance; in addition the rate of acceleration is higher than in the standard procedure. Thus this protocol is considered to be more sensitive to detect defects in motor coordination and balance than the standard protocol (Contet et al., 2001). Nine pairs were tested in London (CMC) and three in Oxford (RD).

### 2.3. Statistical analysis

Experimental results were compared between patient- and control-IgG injected animals using paired *t*-tests. Correlations were calculated with Pearson's correlation coefficient (*r*). The differences between values were considered to be significant when the *p* value was below 0.05. Bonferroni correction was applied to account for multiple testing. We used Graphpad InStat and Prism 4 (GraphPad Software Inc., San Diego, CA) to calculate summary statistics, *t*-tests and correlations, and to create the figures.

## 3. Results

No weight changes were observed in either CRPS- or control-injected mice over the 4 days of the experiment (not shown). There was no overt sickness in the mice and general behaviour in the cages was normal except following the ip injections when there was a tendency to stretch and arch the back (presumably because of discomfort from the one ml of IgG injected). This always resolved within 20 min. No CRPS-typical limb swelling or colour changes were observed upon visual inspections between days 2 and 5.

**Table 1**  
Summary of patients' baseline characteristics ( $n = 12$ ).

Patient no.	Age (years)	Sex	Initiating trauma	CRPS type	Limb affected	Disease duration (months)	CRPS signs	NRS screening	relative pain
1	44	F	1	1	L	16	1, 2, 3, 4	8.3	43 <sup>b</sup>
2	44	F	3	1	U	6	1, 2, 3, 4	9	133
3	39	M	2	1	U–L	18,10	1, 3, 4	7	114
4	24	F	3	1	U	28	1, 2, 3	6.3	108
5	36	F	1	1	L	29	1, 2, 4 <sup>a</sup>	8.7	100
6	50	F	3	1	U–U–L	16 years, 34, 22	1, 2, 3, 4	9	50 <sup>b</sup>
7	55	M	3	0	U	30	1, 2, 3, 4	10	90
8	52	F	3	1	U	12	1, 2, 4	6.9	93
9	38	M	1	1	L	12	1, 2, 3, 4	7.9	69 <sup>b</sup>
10	47	F	2	1	U	23	1, 2, 4	6.6	0 <sup>b</sup>
11	47	F	2	1	L	22	1, 3, 4	7.3	71 <sup>b</sup>
12	32	F	1	1	L–L	10 years, 12	1, 2, 3	8.5	100

No. = Patient enrolment number; F = female, M = male; 'Initiating trauma': 1 = none or trivial; 2 = trauma without fracture; 3 = fracture or operation; 'CRPS Type': 0 = not determined, 1 = without nerve injury; 'Limb affected': L = lower limb – U = upper limb; 'CRPS signs' on first presentation, 1 = allodynia and/or hyperalgesia, 2 = temperature and/or colour abnormality, 3 = swelling and/or sweating abnormality, 4 = motor and/or trophic changes.

<sup>a</sup> 4 = dystonia; 'NRS screening': Average of 7 pain diary entries on a 11 point Numeric Rating Scale (0 = no pain; 10 = pain as bad as you can imagine) taken during a 7-day screening period; N/A = not applicable; Relative Pain: Pain intensity over a 14 day period after IVIG, expressed in percent of the pain intensity after Saline treatment.

<sup>b</sup> Considered a significant IVIG effect. Clinical data from (Goebel et al., 2010).

**Table 2**  
Rearing in the open field.

Experiment No.	1	2	3	4	5	6	7	8	9	10	11	12	Σ
CRPS-IgG group rearing (%)	68*	80	66*	73	55*	66	85	114	81	74	65	120	80**

Groups of 6–10 mice were injected with serum-IgG (~17 mg) derived from either patients with CRPS or healthy volunteers, and tested over 3 min for their rearing in an open field. In each of twelve experiments, relative rearing was calculated as the rearing of the CRPS-IgG injected rodent rearing in percent of the respective control-IgG injected group; numbers 1–12 correspond to the patient numbers in Table 1; CRPS group rearing was lower in 10/12 group pairs (statistically significant in pairs 1 and 5 \* $p < 0.02$  for each, and pair 3  $^{\dagger}p < 0.04$ ); in the pooled analysis of all twelve experiments (Σ) rearing was significantly lower in the CRPS-IgG injected animals (\*\* $p = 0.0004$ ).

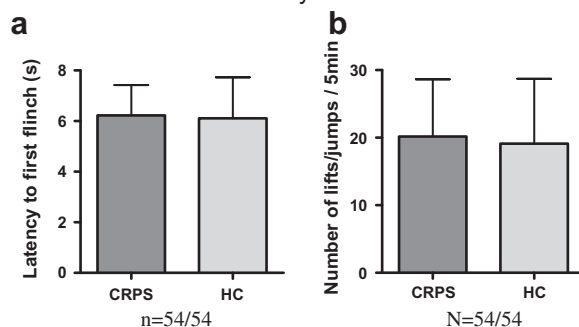
There were no obvious signs of distress during the open field tests (Blackburn-Munro, 2004). The open field results of injection of 12 CRPS-IgGs were compared with 12 control-IgGs (Pairs 1–12) and the results shown in Table 2; the raw data are given in the supplementary appendix (Supplementary data Table S1. See the online version at doi:10.1016/j.ejpain.2010.10.005). On one occasion the exploratory activity of both CRPS and control groups was reduced (Supplementary data Table S1, pair 6) probably as a result of a failure of the air-conditioning. Baseline rearing in the control groups was variable, ranging between 3 (in pair 6) and 30 rears per 3 min period. In three of the twelve experimental pairs (nos. 1, 3 and 5), the number of rears in the CRPS-IgG injected groups were significantly lower than those in their respective control groups (Table 2); the reverse was not observed. Overall, the rearing in the CRPS-IgG injected cohort (mean 17.9,  $n = 84$ ) was significantly reduced compared with that in the control-IgG injected animals (mean 22.2,  $n = 83^{\dagger}$ ; mean of differences 4.2, confidence interval –6.5 to –2,  $p = 0.0004$ , Supplementary data, Table S1).

There were no significant changes in the other open field parameters, including latency to the first rear (average CRPS/Control-IgG: 23.3/20 s,  $p < 0.3$ ), crossings (average CRPS/Control-IgG: 80.4/87,  $p < 0.2$ , Supplementary data, Table S1).

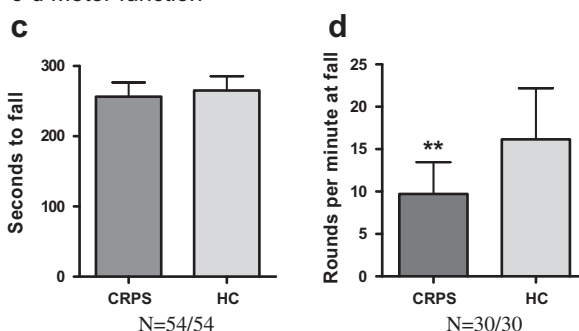
Mechanical allodynia with von Frey hairs was tested in experimental pairs 5 and 6, and the results showed no differences between test and control (Supplementary appendix, Table S2. See the online version at doi:10.1016/j.ejpain.2010.10.005). Nine of the 12 pairs were tested for behavioural responses to heat or cold stimuli. Withdraw-latencies to heat were not different between all IgG injected groups (average CRPS-IgG/Control IgG: 6.3 s/6.1 s, Fig. 1a). Cold sensitivity was less homogeneous between test-pairs, but there were no overall differences between CRPS-IgG and Control-IgG values (average CRPS-IgG/Control-IgG: 20.2/19.1 lifts/jumps, Fig. 1b). Heat and cold sensitivity experiments were stopped for animal welfare reasons when interim analysis after testing of nine group pairs showed no differences between patient and control groups (interim analysis after six group pairs had suggested the possibility of enhanced cold sensitivity in the CRPS-IgG injected animals).

We tested all groups for global motor function and coordination on a rota-rod. In nine test-pairs a standard protocol was used as previously described (Zimmermann et al., 2007), and no significant differences between CRPS-IgG and control-IgG were observed ( $n = 108$ , mean time to fall from rod: 256 s vs. 265 s, Fig. 1c). In the preparations from patients 2, 7 and 9, and their controls, a more sensitive protocol was employed (see Supplementary methods section. See the online version at doi:10.1016/j.ejpain.2010.10.005) (Contet et al., 2001). Here the CRPS-IgG injected animals were found to fall off the rod at a significantly lower rotational speed than the control group ( $n = 60$ , 9.7 rpm vs. 16.2 rpm, mean of differences –6.4, confidence interval: –8.8 to –4,  $p < 0.0001$ , Fig. 1d), with significant patient-control difference in each of the three tests (not shown). These differences were attributed in part

#### a-b Heat and cold sensitivity



#### c-d Motor function



**Fig. 1.** Sensitivity to temperature and motor function. (a and b) There was no difference between groups in the sensitivity to temperature stimuli: (a) latency to the first paw lift after heat application in the hargreaves apparatus, and (b) the number of lifts or jumps on a 0° cold plate over 5 min; (c and d) Animals injected with nine preparations of CRPS-IgG fell off the rota-rod at similar times as those in the control groups (c), but in the three remaining CRPS-IgG groups (d), which had been tested with a more sensitive rota-rod protocol, the animals fell at a significantly lower rotational speed than control animals (\* $p < 0.0001$ ). Error bars show the standard deviation. HC = healthy control;  $N = x/y$ : number of animals tested in the CRPS-IgG/healthy control-IgG injected groups.

to an increased rate of very early falls off the rod (followed by replacement). Individual results for all test-pairs for open field behaviour, stimulus-evoked pain and motor function are shown in the supplementary appendix (Table S1). We adjusted the positive test results to account for multiple testing (seven tests, rearing, crossing, latency, heat or cold allodynia/hyperalgesia, classical and adapted rota-rod function). The results remained significant ( $p < 0.003$  for decreased rearing,  $p < 0.0001$  for earlier falls in the adapted rota-rod protocol).

To see whether the experimental results related to the clinical characteristics of the patients or their response to IVIG, each patient's pain intensity before treatment and the relative efficacy of intravenous immunoglobulin treatment in the clinical trial (Table 1) were compared with the passive transfer results. There was no evident relationship between baseline pain intensity, or the treatment response of a patient, and the behavioural depression induced by his/her purified IgG (pain intensity  $r < 0.01$ ,  $p = 1$ ; treatment response  $r = 0.2$ ,  $p < 0.6$ ).

<sup>†</sup>The open field behavioural results of one mouse in the control group on day 2 were excluded from further analysis because of unplanned, short-termed noise intrusion which resulted in cessation of movement. There were no dropouts for any of the other tests.

There were no significant differences between the depression of rearing elicited by IgG preparations from patients with spontaneous onset CRPS ( $n = 4$ ) and others, or with spreading disease ( $n = 3$ ) and others (not shown).

#### 4. Discussion

To look for evidence of pathogenic IgG antibodies in human CRPS sera, we tested the effects of intraperitoneal injections of serum-IgG from CRPS patients and healthy controls on mouse behaviour. We observed no autonomic signs of CRPS or evidence of increased pain sensitivity, but the CRPS preparations induced significantly reduced rearing<sup>2</sup> and rota-rod performance. These findings support the idea that IgG serum auto-antibodies contribute to the pathophysiology of CRPS and that the role of such antibodies should be studied using extensions of this passive transfer model.

Passive transfer of antibodies from humans to mice is an established protocol for demonstrating the pathogenicity of autoantibodies (Toyka et al., 1975). Antibody transfer experiments are often successful despite inter-species differences, probable because the evolutionarily-conserved antigens show substantial homology, and because a variety of species-specific behaviours can be used to study the mice. A pro-nociceptive effect of the transferred IgG would have been conclusive, but we did not find convincing evidence of increased nociception. However, we also measured rearing in the open field because this is a well-studied mouse-specific behaviour and was reduced in our previous study (Goebel et al., 2005a). Rearing is the response to a novel environment (Zlomuzica et al., 2008), and reduced rearing can reflect adverse (including painful) situations (Mills et al., 2001; Teeling et al., 2007) or be due to a direct effect on brain centres which control this behaviour (Inoue et al., 1996; Zlomuzica et al., 2008; Cerbone and Sadile, 1994). Further studies should investigate whether reduced rearing could relate to non-painful complaints in patients, such as fatigue or depression (Mense and Schiltenswolf, 2010). Interference with motor function is another potential explanation for the observed reduction in rearing. Disturbed motor function is one of the four cardinal signs of CRPS (Harden et al., 2007) and pain-independent difficulty with the performance of fine motor movements in the affected limb is characteristic (Maihofner et al., 2007; Moseley, 2004); this is distinct from the other recognised motor signs of weakness, dystonia, tremor and myoclonus (Veldman et al., 1993). We observed impairment of motor activity/coordination in the three group-pairs tested with a modified, more sensitive rota-rod protocol, while locomotion in the open field was not different from controls and the performance of CRPS-IgG injected mice was not different when we used a classical rota-rod protocol. That protocol stipulates replacement of those mice falling off the rod within the first minute, without adjustment of the final result for such early fall. In contrast, the modified protocol adjusts the results for early falls. Furthermore, after an initial phase with stable rotational speed, the more sensitive protocol requires adaptation to a higher rate of acceleration (20 rpm/min) than the classical protocol (10.7 rpm/min, see [Supplementary methods section](#)). Thus the selective dysfunction with the sensitive rota-rod protocol may indicate reduced capacity of the CRPS-IgG injected groups to achieve coordination between somatosensory input and motor output, which is partially reversible by practice, in line with some clinical findings (Maihofner et al., 2007). Additional study is required to detail this defect.

Our serum samples were taken from patients who subsequently received low-dose intravenous immunoglobulin treatment in a clinical trial, which showed overall efficacy of this treatment to

duce pain (Goebel et al., 2010). Since clinical responsiveness to intravenous immunoglobulin is considered circumstantial evidence for an autoimmune pathophysiology, and as not all CRPS are necessarily autoimmune, we had expected that there would be a relationship between the serum-IgG effects in the model and the patients' responses to intravenous immunoglobulin (Rose and Bona, 1993). The observed lack of correlation could be partly because some patients with the highest IgG activity (patients 3 and 5) did not show a response to IVIG because they required the higher doses (2 g/kg) used in most intravenous immunoglobulin-responsive disorders (Dalakas, 2008).

We do not know the target of the putative IgG antibodies in CRPS or the immune mechanisms involved, although recent evidence suggests binding to both autonomic and somatic nerves (Goebel et al., 2005b; Kohr et al., 2009). Since abnormal rearing was observed as early as 29 h after IgG injection there may be direct functional effects of the IgG at the antigenic targets (Buckley and Vincent, 2005). Observation of signs relating to more slowly evolving immune mechanisms might have been precluded by the limited duration of our study.

The study was observer blinded and we used preparations from a typical group of patients with longstanding CRPS (although recruitment bias cannot be excluded). There was variability in the control results, but all experiments were done in pairs of CRPS and control, so confounding factors should not impact the overall results. We did not attempt to demonstrate anti-neuronal autoantibodies in our patients. Currently there is no commercially available serum test to allow standardised testing for the recently described CRPS serum activity *in vitro* (Kohr et al., 2009); anti-sympathetic activity has been reported to occur in about 50% of early CRPS (<6 months disease duration), but its prevalence in later CRPS is unknown (Kohr et al., 2009). A more important limitation is that our model did not involve limb trauma. CRPS is a posttraumatic condition in 90% of cases (Veldman et al., 1993), and serum autoantibodies may exert their role mainly in the context of an altered environment after trauma. Future models should now entail the application of a minor peripheral limb trauma before or after the IgG transfer.

In summary, we demonstrate here for the first time that IgG transfer from patients with longstanding CRPS causes abnormal rodent behaviour and motor function. These results support a pathophysiological role of CRPS serum-IgG and lend further credence to the idea that CRPS, at least in some cases, has an important autoimmune component. In the absence of limb trauma, IgG transfer does not produce the typical CRPS limb aspect or hyperalgesia and is therefore not a suitable model for CRPS.

#### Declaration of interest

Dr. Goebel has received grant support from CSL-Behring, Bern, Ch, and Talecris, USA and speaker honoraria from Baxter, USA. The other authors declare no conflict of interest.

#### Acknowledgements

The authors wish to thank Mrs. Gabrielle Turner and Dr. Bjarke Abrahamsen for technical assistance, Prof. John Wood for technical advice and help with the initial study design, and the staff of the clinical laboratory, Queen Square for generous support. The work was supported by a grant from the Association of Anaesthetists in Great Britain and Ireland (AAGBI). C.M. Cendán was supported by the Research Program of the University of Granada. The funding bodies had no role in the conduct of this research.

<sup>2</sup> Rearing: the number of times the mouse stands completely on its hindlegs.

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## Appendix A. Methods

### A.1. Behavioural tests

All tests were approved by the United Kingdom Home Office Animals (Scientific Procedures) Act 1986 and performed in a Home Office designated room at  $22 \pm 1$  °C.

#### A.1.1. von Frey hairs

Mice were acclimatized in the test chambers for at least 1 h until exploratory activity had ceased. Hairs were applied three times each, through a mesh floor, until a response was elicited (paw withdrawal, shaking or biting) using the up–down paradigm (Abrahamsen et al., 2008). Results were expressed as the paw withdrawal threshold to mechanical stimulation for each group  $\pm$  the standard error of the mean (SEM).

#### A.1.2. Hargreaves' test

Mice were acclimatized in the test chambers for at least 1 h until exploratory activity had ceased. The latency of withdrawal to heat was recorded 4–6 times on each paw, with at least 1 min

between recordings on the same paw (Abrahamsen et al., 2008). Results were expressed as the mean withdrawal latency for each group  $\pm$  SEM.

#### A.1.3. Cold plate test

Responses to cold were assessed using a cold plate at 0 °C monitoring paw lifts or jumps during 5 min (Zimmermann et al., 2007). Results were expressed as the mean of lifts or jump for each group  $\pm$  SEM.

#### A.1.4. Rota-rod test (standard procedure)

Mice were placed on the rota-rod (Jones and Roberts, 1968) as it was rotating at 20 rpm. After 1 min the rate of revolution was increased, and reached a maximum of 36 rpm within 90 s. The length of time that each animal spent on the rod was measured, with a cut off time of 5 min. Animals which fell off the rod within the first minute were replaced without adjustment for the fall. Results were expressed as the mean time spent on the rod for each group  $\pm$  SEM. The more sensitive rota-rod procedure, used in three experiments is described in the main text.

**Table S1**  
Results of behavioural tests.

No.	Number of animals tested		Number of rears		Number of crossed fields		Latency to first rear (s)		Hargreaves <sup>a</sup>		Cold plate <sup>b</sup>		Rota-rod <sup>c</sup>	
	P	C	P	C	P	C	P	C	P	C	P	C	P	C
1	6	6	17	25	78	112	9	12	6	6	31	28	262	258
2	10	10	17	21	80	79	42	26	–	–	–	–	9 <sup>d</sup>	17 <sup>d</sup>
3	6	6	20	30	93	128	32	15	6	6	21	28	221	237
4	6	6	19	26	62	74	25	30	6	5	15	14	255	270
5	6	6	15	27	82	88	–	–	7	7	23	16	283	295
6	6	6	6	9	66	64	–	–	9	10	25	19	241	234
7	10	10	17	20	79	80	27	18	–	–	–	–	9 <sup>d</sup>	14 <sup>d</sup>
8	6	6	33	29	130	121	10	12	5	6	27	22	239	261
9	10	10	17	21	80	96	25	28	–	–	–	–	11 <sup>d</sup>	18 <sup>d</sup>
10	6	6	14	19	70	71	45	27	6	5	13	16	260	270
11	6	6	15	23	71	69	17	13	6	5	15	19	264	281
12	6	6	24	20	74	56	15	22	5	5	12	12	282	281

Rodent behaviour after IgG transfer. No. = Patient enrolment number. P = Patient IgG fraction; C = Control IgG fraction; – = Measurement not taken.

<sup>a</sup> Heat allodynia/hyperalgesia tested in a Hargreaves apparatus (the numbers denote seconds to first flinch).

<sup>b</sup> Cold allodynia/cold hyperalgesia tested on a cold plate (number of jumps or flinches).

<sup>c</sup> Motor coordination on a rota-rod (seconds or <sup>d</sup> rounds per minute: a different, more sensitive protocol of assessing motor coordination on the rota-rod was employed in these three experiments).

**Table S2**  
Mechanical allodynia to von Frey hairs in experimental groups 5 and 6.

	P	HC
Baseline 5	1.0 $\pm$ 0.3	1.3 $\pm$ 0.2
Post-injection 5	0.7 $\pm$ 0.1	0.9 $\pm$ 0.1
Baseline 6	0.9 $\pm$ 0.1	1.0 $\pm$ 0.2
Post-injection 6	1.1 $\pm$ 0.3	1.1 $\pm$ 0.2

The numbers denote the baseline and post-injection paw withdrawal threshold  $\pm$  the standard error to mechanical stimulation with calibrated von Frey hairs expressed in grams in animals treated with patient (P) and healthy control (HC) IgG. No statistically significant differences between the two groups were observed (*t*-test). 5/6 = experimental groups 5/6.