Deelproject aanvraag

Projectnummer: -201
Ontvangst: 15-01-2010

I Project

Onderzoek

- *Aanvrager*

Naam	
Organisatie/Eenheid	
Onderafdeling	
Adres	
Postcode	
Plaats	
Telefoon	
E-mail	
Vergunninghouder	: [1] RUG

- *Algemeen*

Titel basisproject	: Modeling the role of myelin/oligodendrocyte glycoprotein in the regulation of immune activation within the central nervous system
Aanvang basisproject	: 01-03-2010
Gewenste duur	: 4 jaar
Voorgaande (deel)project(en)	:
Geldstroom	: [5] combinatie
Subsidiegever	:

- *Eind- of medeverantwoordelijke*

Titel en naam	

- *Andere personen betrokken bij de handelingen aan proefdieren*

Naam	Functie

Deelproject

- *Algemeen*

Titel deelproject	: Development of EAE in human DC-SIGN transgenic mice
Aanvang deelproject	: 01-03-2010
Gewenste duur	: 1 jaar

II Onderzoeksopzet

- *Behaalde resultaten*

Chronic autoimmune disease is a pathological hyper-responsiveness of the immune system to damage in a target organ. The disease of our interest is multiple sclerosis (MS), which is modelled in experimental autoimmune encephalomyelitis (EAE). Our work in a unique EAE model in the non-human primate "common marmoset" has shown that T-cells against MOG have a key pathogenic role in the development of neurological deficits . The aim of our research is therefore to analyse the regulation of T-cell tolerance and immunity against MOG.
The underlying hypothesis has been formulated in the Yin/Yang paradigm . Essentially, this paradigm states that the induction of T-cell tolerance or (auto)immunity depends on the maturation state of the antigen presenting cell (APC). APC maturation is regulated by the integration of input signals relayed via innnate receptors, i.e. activating signals via Toll-like receptos (TLR) and inhibitory signals via C-type lectin receptors (CLR). Work by van Kooyk et al. has clearly shown that antigen binding and uptake via inhibitory CLR, of which DC-SIGN is a thoroughly investigated example, inhibits APC maturation leading to T-cell tolerance.
Where do these processes take place? In the course of EAE myelin debris released from injured myelin sheaths in demyelinating lesions are drained to the cervical (for the brain) and lumbar (for the spinal cord) lymph nodes. There myelin can be found within APC with an immature dendritic cell-like phenotype expressing the CLR DC-SIGN+ve. The localization of these cells within areas where T-cells are activated is highly suggestive for antigen presentation to myelin reactive T-cells. Indeed, surgical removal of cervical and lumbar lymph nodes from Biozzi ABH mice impairs the characteristic chronic relapsing EAE course of this strain in response to recombinant MOG (van Zwam, J. Pathol. 2009).
Conceptually, MS patients can be regarded as genetically-predisposed high responders to myelin that is released from injured CNS as consequence of an unknown primary pathogenic event . We have analysed in marmoset monkeys against which specific myelin component the autoreactive T-cells that induce CNS injury and neurological deficit are directed, i.e. an epitope encompassed within peptide 40-48 of MOG .

- *Vraagstelling van het deelproject*

We would like to set-up an experimental model in mice to test the hypothesis that T-cell tolerance is induced when the conditions for interaction of normally glycosylated MOG (Lewis x +ve) with DC-SIGN are present, whereas T-cell immunity is induced when this interaction is impaired.

- *Onderzoeksopzet*

The series of experiments will be conducted using wild-type WT C57BL6 and 3 genetically modified strains:
-DC-SIGN transgenic (Tg) C57BL6 mice will be used to model the restricted expression of the CLR in humans. We will get these mice from [redacted]
-We will use MOG-deficient (MOG-/-) C57BL6 mice generated by Dr. Pham-Dinh in Paris. We will get these mice from our collaborator [redacted]
-We will use Mgat5-deficient mice. Mgat 5 encodes the glycosyltransferase 5, which catalyzes the formation of DC-SIGN binding carbohydrates. These mice develop an SLE-like multi-organ autoimmune disease with ageing (Demetriou, Nature 2001). We will get these mice from the Consortium of Functional Glycomics via our collaborator [redacted]
We will test:
* Whether WT C57BL6 mice immunised with myelin from WT mice develop EAE (control). We predict that they will develop EAE (as demonstrated in Smith, Eur J Immunol 2005, 35: 1311-1319)
* Whether DC-SIGN Tg C57BL6 mice immunised with myelin from WT mice develop EAE. We predict that they will be tolerant to EAE
* Whether WT C57BL6 mice immunised with myelin from MOG-deficient mice do develop EAE. We predict that they will not develop EAE (as demonstrated in Smith, Eur J Immunol 2005, 35: 1311-1319)
* Whether DC-SIGN Tg C57BL6 mice immunised with myelin from MOG-deficient mice do develop EAE. We predict that they will not develop EAE
* Whether WT C57BL6 mice immunised with myelin from Mgat5-deficient mice develop EAE. We predict that they will develop EAE
* Whether DC-SIGN Tg C57BL6 mice immunised with myelin from Mgat5-deficient mice develop EAE. We predict that they will develop EAE as abnormally glycosylated MOG cannot bind to DC-SIGN.
Groups:
1 WT myelin in WT mice†10Female
2 WT myelin in DC-SIGN Tg mice†10Female
3 MOG-/- myelin in WT mice†10Female
4 MOG-/- myelin in DC-SIGN Tg mice†10Female
5 Mgat5 -/- myelin in WT mice†10Female
6 Mgat5 -/- myelin in DC-SIGN Tg mice†10Female
So, the EAE experiment will comprise 60 mice in total, i.e. 30 WT mice and 30 DC-SIGN transgenic mice.

For myelin preparation 60 WT mice, 60 MOG-deficient (MOG-/-) mice and 60 Mgat5-deficient mice (Mgat5 -/-): to immunize 10 mice, 40 myelin donor mice are needed of at least 12 weeks old.

- *Beschrijving van de uitvoering van de experimenten*

We will use the standard immunization protocol for EAE induction in mice:
Briefly, animals will be injected subcutaneously (s.c.) into two sites on the flanks with a sonicated emulsion consisting of either 200 Ag peptides or 1 mg SCH added to PBS, emulsified in complete Freund's adjuvant (CFA) on post-sensitization day (PSD) 0 and 7. Myelin- immunized mice will also be injected with pertussis toxin, (200 ng) derived from Bordetella pertussis (Sigma, Poole, UK) dissolved in PBS and administered intraperitoneally (i.p.) on PSD 0, 1, 7 and 8. Mice will be weighed and graded daily for neurological signs according to the following scale: Grade 0—No disease; 1—Limp tail; 2—Impaired righting reflex; 3—Partial hindlimb paralysis; 4—
Complete hindlimb paralysis; 5—Complete forelimb paralysis/moribund.

For ethical reasons mice will be sacrificed within 48 hours after score 4 EAE has been reached. The mice will be sacrificed.
The post mortem analysis will comprise:
-profiling of the immune status. To this end spleen and lymph nodes will be aseptically removed and processed for preparation of mononuclear cell suspensions. These will be cultured with relevant antigens to investigate the immune status.
-brain and spinal cord will be removed and processed for immunohistochemical and histological analysis

- *Ongerief voor dieren*

Handeling	Ongerief
Humane doding tbv isolatie hersen en ruggemerg	[1] gering
Inductie van EAE door inoculatie van antigeen/adjuvant emulsie plus intraveneus Bordetella De belangrijkste oorzaak van ongerief is de immunisatie met bacteriële adjuvantia. Deze veroorzaken ontstekingsreacties rond de injectieplaatsen, i.e subcutane injecties in de flank, en kunnen koorts veroorzaken. Het is onmogelijk dit te reduceren aangezien de ziekte zonder zonder deze adjuvantia niet reproduceerbaar kan worden opgewekt. EAE is geassocieerd met het verlies van sensorische en motorische functies wat inherent is aan de aard van de gemodelleerde humane ziekte. Pijn treedt niet aantoonbaar op. Dit ongerief wordt over het algemeen als matig beoordeeld.	[5] ernstig
Dagelijkse beoordeling van neurologische defecten De dieren worden daarvoor uit hun kooi gehaald, gewogen en de aanwezigheid van symptomen wordt beoordeeld volgens gestandaardiseerd klinische score protocol (score van 0 tot 5)	[3] matig

Totaal ongerief deelproject

[5] ernstig

Toelichting

Handeling
Mice that will be sacrificed for preparation of myelin will experience only minor discomfort.
The immunization procedure with complete Freund's adjuvant is regarded as serious discomfort.
The daily inspection for neurological signs: mild discomfort
EAE is associated with loss of neurological functions that may cause some stress, but pain is not present: moderate discomfort
For the EAE experiment: serious discomfort; this grade is due to the usage of complete Freund's adjuvant (CFA) for the induction of reproducible disease.
Toelichting:
The adjuvant causes granulomatous ulcerations at the injection sites. There is still no effective alternative for this adjuvant.

Verfijning van uitvoering

CFA cannot be replaced.
The mice will be sacrificed within 48 hours after score 4 EAE has been reached with the aim to limit the period of clinically active disease.

- *Dieren: soort(en) en aantal(len) voor de periode van dit deelproject*

Diersoort	Stam	Inteelt	Aantal
Muizen	C57BL6 wild type	Inteelt	90
Muizen	C57BL6 huDC-SIGN	Transgeen (tevens inteelt)	30
Muizen	C57BL6 MOG -/-	Knock-out (tevens inteelt)	60
Muizen	C57BL6 MgatV -/-	Knock-out (tevens inteelt)	60

Motivering aantal dieren

As the disease incidence is not always 100% a standard group size of 10 is used to maximize the cahnce that resultswill be obtained that can be statistically tested.
Groups 1 to 6 will contain 10 mice each. We always include the risk of at least two outliers in each group, for example because the EAE incidence can vary between 80 and 100%. Results will be given as survicval curves and statistical evaluation will be done with Fischer exact test. According to the 2 x 2 table given below, 10 mice per group result in a sufficiently powered study.

Exposed p1 (exposed)1,000
Effect Yes No Total SE p1 0,000
Yes 10 2 12 p0 (control) 0,200
No 0 8 8 SE p0 0,126
Total 10 10 18 Diff p1 - p0 0,800
SE Diff p1 - p0 0,126
Diff (CI 95%) 0,80 (0.53 - 1.07)
c2 uncorrected 13,333
p value (uncorrected) 0,0002607
c2 Yates correction 10,208
p value (Yates correction) 0,001
Fishers exact test 0,000
Odds Ratio (CI 95%) #VALUE!
Relative Risk (CI 95%) 5,00 (1.45 - 17.27)
Statistical Power
Observed Power (1-b) at a = 0,000 0,955
Number required at 1-b = 0,01 ; a = 0,001 12
Number exposed/treated 6
Number controls 6
Required Difference |p1-p0| 0,614

Set a 0,05
Set 1-b 0,80

Vermindering van aantallen

There is no room for reduction of animal numbers as this will affect the power of the study

III Proefdierkundige gegevens

- *Dieren: herkomst en huisvesting*

Herkomst van dieren

[1] aankoop

Toelichting herkomst van dieren

- wild type mice will be purchased from commercial breeders (Harlan).
-DC-SIGN transgenic (Tg) C57BL6 mice will be purchased from
-MOG-deficient C57BL6 mice will be purchased from
-Mgat5-deficient micewill be purchased from the Consortium of Functional Glycomics

Dieren uit ander onderzoek

n.v.t.

Afwijkende huisvesting

n.v.t.

Locatie van de experimenten

[5] CDL

Elders

n.v.t.

- *Anesthesie en pijnbestrijding*

Anesthesie

[4] wordt toegepast

Welke of waarom niet

isofluraan

Pijnbestrijding (per- en) postoperatief

[1] wordt niet toegepast (geen aanleiding)

Zo ja: Welke

Diersoort	Medicament	Dosering (mg/kg)	Route	Interval (uur)

Eventuele andere pijnbestrijding

-

Toedieningsschema: frequentie en periode

-

- *Einde experiment*

Toestand dier na proef

[1] dood in kader van de proef

Indicaties humane eindpunten

Muizen die EAE score 4 bereiken worden zo snel als mogelijk, maar maximaal na 48 uur opgeofferd. 0: geen EAE verschijnselen, 1: slappe staart of langzame oprichtingsreflex, 2: staartverlamming en milde achterpootzwakte, 3: matige achterpootzwakte, 4: paraplegia maar normaal gebruik voorpoten, 5: paraplegia en voorpootzwakte, 6:stervend. Voor de muizen in proef wordt gelet op algemene welzijnsparameters en wordt ingegrepen (opoffering) wanneer er zich onverwachte zaken voordoen.

Wijze van termineren

Onder isofluraan anaesthesie gebrachte muizen worden via cervicale dislocatie getermineerd

IV Biotechnologie

Type	Nummer en specificatie