

1 1. Supplementary materials and methods

2 1.1. *In vitro* analysis of immunocompatibility between recipient spleen and lymph node
3 cells and donor Schwann cells via [3H]thymidine incorporation assay

4 Since sufficient numbers of neonatal rat Schwann cells for this study were not obtainable
5 from Lewis LEW/OrlRj breeds in reasonable time (small litter sizes and low proliferation
6 rate of primary cells), we decided to use neonatal Schwann cells from Wistar RjHan:WI
7 breeds. The transfer of genetically modified SCs derived from Wistar RjHan:WI rats within
8 CNGs into the recipient LEW/OrlRj rats displays, however, an allogenic transplantation,
9 which comprises the risk of an immunoreaction and transplant rejection.

10 With the supplementary material to this study, we provide data on our evaluation of the
11 probability for an immunoreaction to occur. Briefly, we performed *in vitro* proliferation
12 assays of recipient Lewis LEW/OrlRj rat lymphocytes, derived from either the spleen (Spl)
13 or the cervical lymph nodes (LN), cultivated with either donor Lewis LEW/OrlRj rat (Lew)
14 SCs, serving as negative control, Wistar RjHan:WI rat (Wi) SCs, serving as experimental
15 group, and Sprague Dawley RjHan:SD rat (SprD) SCs, serving as additional experimental
16 group. And with this we could demonstrate that the probability to induce a host-versus-
17 graft response with transplanting RjHan:WI-derived cells into LEW/OrlRj rats is close to
18 zero (supplementary figure 1).

19 1.2. Preparation of donor Lewis LEW/OrlRj, Wistar RjHan:WI, and Sprague Dawley
20 RjHan:SD rat Schwann cells

21 Lew, Wi, and SprD SCs were harvested and cultured as described in section 3.1. SCs at
22 passages at 10-12 were prepared to conduct the following experiment. SCs were counted
23 and 5.000 SCs were seeded into each well of a 96 well plate (Nunc, Thermo Fisher
24 Scientific). For each strain 9 wells of a 96 well plate (Nunc, Thermo Fisher Scientific) were
25 seeded with 5,000 SCs each. 3 of the 9 wells per strain remained with solely SCs, to other
26 3 wells $2 \cdot 10^5$ Spl or LN cells were added. Cells were incubated for 5 days at 37°C with
27 5% CO₂. On day 4 0.5–1 µCi [3H]thymidine per well was added for another 16 hours.

28 1.3. Preparation of recipient Lewis LEW/OrlRj rat lymphocytes

29 Two female adult Lewis rats were transferred into carbon dioxide atmosphere for inducing
30 in deep anesthesia and killed by cervical dislocation. The spleen, as well as the cervical
31 lymph nodes were explanted and chopped up. Afterwards the pieces of the spleen, as
32 well as the pieces of the lymphnodes were separately meshed through a strainer.
33 Afterwards, a lysis buffer on NH₄Cl basis was used to remove erythrocytes. Spleen and
34 lymphnode cells were counted and a total amount of $2 \cdot 10^5$ cells was added to each well
35 seeded with SCs as described in section 3.6.1.

36 1.4. Proliferation of spleen or lymph node cells – [3H]thymidine incorporation assay

37 For detecting the proliferation rate of stimulated Lew Spl or LM lymphocytes with either
38 Lew, Wi, or SprD SCs, 0.5–1 μ Ci [3H]thymidine per well was added on day 4. After 16
39 hours the [3H]thymidine incorporation was assessed after szintilisation using a β -counter
40 (LKB Wallac, Turku, Finland).

41 2. Supplementary results of the *in vitro* analysis of immunocompatibility between
42 recipient spleen and lymph node cells and donor Schwann cells via [3H]thymidine
43 incorporation assay

44 [3H]thymidine incorporation values of wells seeded with solely Lew, Wi, or SprD SCs were
45 subtracted from respective values obtained from wells seeded with Lew, Wi, or SprD SCs
46 and Spl or LN cells from adult Lew rats to obtain the values shown in supplementary figure
47 1. Additionally, baseline reference values for solely Lew Spl and LN cell proliferation were
48 obtained.

49 The stimulation of Lew Spl cells with Lew SCs resulted in $6985,67 \pm 1427.88$ counts per
50 minute (CPM), followed by the stimulation with Wi SCs, leading to 10969.67 ± 4896.01
51 CPM. The highest proliferation of Spl cells was induced by the SprD SCs with 20035.50
52 ± 16556.90 CPM. Values for all 3 groups exceed the baseline values, obtained for Spl cell
53 proliferation without any SC induced stimulation. An overall lower [3H]thymidine
54 incorporation was observed for the stimulated Lew LN cells when compared to the Lew
55 Spl cells. Interestingly, stimulation of Lew LN cells with Lew (-1468.17 ± 1572.37) as well
56 as Wi SCs (136.34 ± 1015.88) resulted in lower proliferation rates when compared to the
57 LN cell proliferation without any SC induced stimulation (1095.00 CPM). The incubation
58 of Lew LN cells with SprD SCs led to the highest [3H]thymidine incorporation with 2866.00
59 ± 4399.61 CPM. The moderately induced proliferation of either Lew Spl or LN cells by Wi
60 SCs allows, in accordance with the animal care committee of Lower-Saxony, Germany,
61 the conclusion that the transplantation of Wi SCs into Lew rats will probably not lead to
62 any immune reaction or transplant rejection *in vivo*.

63 3. Supplementary figure captions

64 **Supplementary figure 1.** Proliferation of recipient Lewis (Lew) spleen (Spl) or lymph node
65 (LN) cells. Dot plot displaying [3H]thymidine incorporation of Lew spleen and lymph node
66 cells after 5 days *in vitro* with donor Schwann cells (SCs) of either Lew, Wistar (Wi), or
67 Sprague Dawley (SprD) rats. Black horizontal line displays the mean solely Lew LN cell
68 proliferation. Grey horizontal line displays the mean solely Lew Spl cell proliferation. While
69 Lew Spl cells are barely stimulated by Lew SCs, Lew LN cells are not stimulated at all.
70 Culturing Lew Spl cells with Wi SCs, results in minor proliferation, while culturing Lew LN

71 cells with Wi SCs does not lead to any stimulation. SprD SCs trigger a high proliferation
72 in Lew Spl cells and a minor proliferation in Lew LN cells. No statistical analysis (n = 2).

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