License Number: GZ: 925665/2013/20

Project Title: "Modulation of Gene Activity in Mice 3 – Immunological Process"

License Holder: Meinrad Busslinger

Number of Mice: 800 (160 WT and 640 GMO) within 5 years

Severity classification according §3 TVG 2012: Mild

License valid until: 04. December 2018

Project proposal and description:

We herewith apply for a new permit for our laboratory animal project in the field of "Modulation of Gene Activity in Mice" successfully performed now for years and in the course of which we specifically influence gene activity to be able for instance to analyse the consequences of lacking a certain gene in the animal and hence its functions. This type of study involving "knock-out mice" has proven to be a central tool of international gene research over recent years.

Knowledge of functions of particular genes in the development of the immune system and immune response is required for instance to be able to develop efficient therapies against cancer and immune diseases. Genome research has revealed the number of genes in model organisms such as the mouse, and also for human beings. Our knowledge of the functions of these genes is however still very poor, in recent years therefore systems allowing targeted activation or deactivation of expression of genes following a time-based or tissue specific pattern have been and continue to be developed. The aim of these experiments is to analyse the expression and function of genes in blood cell differentiation, immune response and tumour development.

During the last experimentation period we successfully performed numerous experiments concerning the function of genes in development of blood cells and leukaemia. The success of these experiments has been documented in enclosed international publications (see enclosure).

The animals involved are inbred strains such as C57BL/6, 129 or CBA. Additionally, animals with mixed strains (such as C57BL/6x129, 129 x CBA) are also used. Examinations of the respective genes and their mutant forms are carried out on the genetic strain of the aforesaid mouse lines.

This project application concerns immunological procedures for targeted activation or deactivation of gene expression.

Immunological Procedure
Some of the genes to be researched which encode transcription factors (Pax5, EBF1, E2A, Ikaros, STAT5, GATA3, RBP-Jk, etc.), recombinases (RAG1/2, AID), cytokines (IL-7, Flt3 ligand), receptors (Notch1, Flt3, IL- 7R) and signal transmission molecules (BLNK, PI3 kinase. Pten), are assumed to play a significant role in modulation of the immune response. The function of these genes is to be

examined using immunological experiment setups. For this purpose the immune system of transgenic animals which have the gene to be examined either over-expressed or deleted are to be activated through application of specific antigens and afterwards examined using conventional immunological methods for its reaction.

Type of Procedure and/or Treatment

Methodology:
The inducible modulation of gene activity will be solely carried out in mice. The functional role of particular genes in differentiation of immune cells, immune response and development of leukaemias are to be researched in detail. Some of these mice will originate from mice lines created within the already approved animal experiment protocols (transgenic, knockout and inducible, genetically modified mice).

Defined T-cell dependent and T-cell independent antigens such as NIP-Ficoll, NP- KLH, NIP-Ovalbumin and polyclonal antigens (e.g. sheep blood cells) are administered to transgenic animals. In order to study particular immune reactions, certain bacteria and viruses (such as vesicular stomatitis virus, VSV) are also used to infect transgenic mice kept in isolation.

Description of the required animals:

Mice of various inbred strains (C57BL/6, CBA, 129, C3H) and of differing sex are used. Adult animals will be used.

Groups of on average 20 experimental animals and 20 control animals, treated with placebos, are required for one experiment.

Housing and feeding: according to standard conditions.

Habituation, preparation and conditioning of the experiment animal: not required.

Treatment: Experiment description and examination of immune responses

Examination targeted at development of germ concentrations in lymphoid organs:
Mice are intraperitonealy immunised with NP-KLH or HEL in alum-adjuvant or sheep erythrocytes (without adjuvant) and euthanized on day 8 to 14, or subcutaneously boosted on day 21 with NP-KLH (without adjuvant), before being euthanized. The spleen and lymph node cells are removed from the euthanized mice and analysed through flow zytometry.

Examination for development of plasma cells:
To study the T-cell dependent immune response the mice are intraperitonealy immunised with NP-KLH or HEL in alum-adjuvant and euthanized on day 21.

The T-cell-independent immune response is induced through intraperitoneal immunisation with NP-Ficoll. The mice are euthanized between day 14 and 21.

In both cases the cells of the bone marrow, spleen and lymph nodes are analysed for plasma cells

through flow zytometry and examined for specific plasma cells using ELISPOT.

Examination of antibody production
T-cell dependent immune response (as described above): A maximum of 100 µl of blood is taken from the mice (on day -7; zero value). On day 1 the mice are intraperitonealy immunised with NP-KLH in alum- adjuvant. Subsequently a maximum of 100 µl blood is taken on each of day 7, 14 and 35. The T-cell-independent immune response (as described above): intraperitoneal immunisation with NP-Ficoll followed by blood sampling as described above.

Both cases provide serum which is analysed through ELISA for specific antibodies of various immunoglobulin classes (isotypes).

The required dosage of immunostimulants to be administered in order to achieve optimal effect is already known through publications by other labs.

100 µg antigen protein in 0.1 ml adjuvant per animal (one injection point). Maximum 2 administrations per animal, with 21 days between. The effects of the administrations and possible effects on body weight and general well-being of the animals is monitored daily.

<u>End-points</u>

End of experiment not later than after eight weeks, however in less than four weeks in most cases. If observable pathological changes appear or other effects impairing the well-being of the animal occur before that point, the animal will be pain-free euthanized before the end of the experimental period.

As this project concerns assessment of the as yet unknown effects of genetic mutations and their function in individual organs or entire organisms, little can be said about the scale of any pathological changes. These are indeed also the subject of this research project. We will therefore apply the general end-points also valid for animals which have not been subjected to any experimental procedures. Visibly sick animals will be handled according to clinical condition determined by veterinary inspection. As a guideline occurrence of the following symptoms counts as an end-point:

Emaciation of animal, acra prominent. BCS 2-1 -> animal is immediately euthanized.

Loss of 20% of body weight -> animal is immediately euthanized. Loss of 10-15% of body weight within 3 days -> animal is immediately euthanized.

Irreversible inability to consume food and water -> animal is immediately euthanized.

Clearly ascertainable breathing problems, clearly impaired breathing, possibly accompanied by inhalation and exhalation wheezing, grinding of teeth, -> animal is immediately euthanized.

Heavy diarrhoea -> animal is immediately euthanized if accompanied by another symptom, otherwise after 4 days.

Clear apraxia, convulsions, cramps, paralyses, tremors, slowed or unresponsive reflexes, amyasthenia or muscle rigidity, ambulation with curved spine, huddled posture, horrent fur, half closed eyes, dragging/swinging of hind quarters (animal is euthanized if there is no improvement four hours after the initial observation.)

Apathy, huddled posture, isolation from other animals, starkly reduced reaction to picking up, starkly reduced reaction to acoustic stimulation and contact, animal cannot be roused -> animal is euthanized if there is no improvement four hours after the initial observation.

Abnormal swellings or protrusions, stark asymmetry of body -> animal is immediately euthanized if symptoms in connection with one or more other symptoms. If the occurrence is not accompanied by other symptoms, further thorough observation of the animal is foreseen, should any additional symptoms occur the animal will be immediately euthanized.

Strong nasal or eye discharge -> animal is immediately euthanized if symptoms are accompanied by one or more other symptoms, otherwise four days after initial observation.

Bleeding from body orifices (light bleeding: animal is euthanized if the bleeding does not abate after 30 minutes. Heavy bleeding: animal is euthanized if bleeding cannot be stopped immediately).

Severe skin lesions -> animal is immediately euthanized if accompanied by one or more other symptoms, or if attempted treatment fails with no prospect of healing, otherwise depending on clinical condition.

Strong aggressive reaction to picking up caused by pain, possibly accompanied by loud vocalisations, gnawing at parts of the body -> animal is euthanized if there is no improvement four hours after the initial observation.

Required post-treatment: none

Euthanasia:

Animals will be pain-free euthanized (cervical dislocation, CO_2) only by qualified staff under supervision of the vet responsible for animal housing. Animal carcasses will be then disposed of when exsanguination has been performed and/or apnoea and cardiac arrest occur (please refer to the application item 22).

Publications:

Malin, S., McManus, S., Cobaleda, C., Novatchkova, M., Delogu, A., Bouillet, P., Strasser, A., and **Busslinger,** M. (2010). Role of STAT5 in controlling cell survival and immunoglobin gene recombination during pro-B cell development. Nat. Immunol. 11, 171-179

Vilagos, B., Hoffmann, M., Souabni, A., Sun, Q., Werner, B., Medvedovic, J., Bilic, I., Minnich, M., Axelsson, E., Jaritz, M_v and **Busslinger,** M. (2012). Essential role of EBF1 in B cell immunity by

controlling the generation and function of distinct mature B cell types. J. Exp. Med. *209, 775-792.*