Summary

Ageing is associated with alterations of gene activities modulating the inflammatory and immune response. Stroke is a common health problem in the European Community and the leading cause of severe long-term disability. Inflammatory reactions within the brain as well as a systemic immune response are assumed to influence the outcome after stroke. We hypothesise that the extent and the progression of the immune response after stroke is altered in elderly patients and that these age-dependent inflammatory reaction is a major determinant of the outcome after stroke. We will characterise the age-dependent inflammatory reaction in brain following stroke by functional genomics and proteomics in the ARGES-project. Furthermore, systemic markers of the inflammatory response after stroke will be identified in circulating leukocytes, which will serve as clinical indicators for potential therapeutical interventions. In addition to the genome-wide approach, we will specifically analyse the functional impact of a pre-selected pathway, the sphingomyelin-ceramide-cycle, considered to be critically involved in stroke-induced destructive processes. The project will substantially contribute to our understanding of the age-dependence of the inflammatory reaction following stroke which has as yet not been sufficiently investigated. We expect that ARGES will deliver indicators for therapeutical interventions whose application may considerable improve the outcome after stroke. We thus will make a significant contribution to basic scientific knowledge, therapy and diagnostics of stroke.

Background

Treatment of brain ischemia has greatly been improved by the possibility of intravenous recombinant tissue plasminogen activator. Unfortunately, this treatment is only possible in the first 3 to 6 hours following stroke, and can be applied to only 5 - 20 % of the patients, even if the logistics to bring the patient into the hospital is organised in an optimal way. Moreover, circumstantial evidence suggests potentially harmful effects of the serine protease rtPA on brain tissue itself. It is therefore an urgent need to find treatment strategies for brain protection extending beyond this early time window.

Unfortunately, numerous strategies for neuro-protection have failed clinically (calcium antagonists, NMDA antagonists, GABA agonists, citicolin, gangliosides, and others). Although

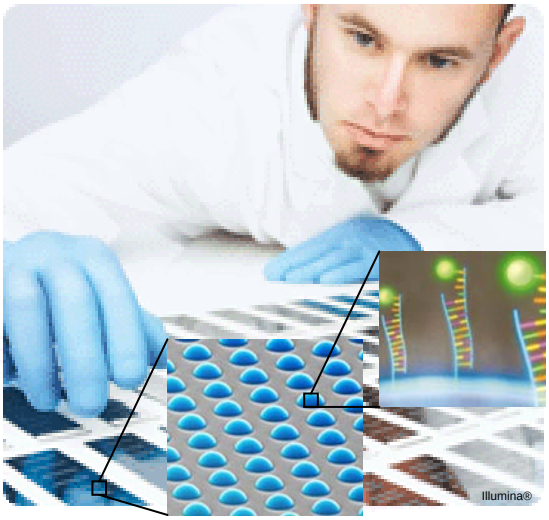
stroke is an extremely important health issue, many pharmaceutical companies have stopped their research programs on brain neuroprotection following stroke due to the negative experience with previous studies.

There are several possible reasons why the previous approaches were not successful. One important reason lies in the fact that the experiments were done with young animals whereas most patients are in older age. There are many indications that the reaction of the brain to an ischemic event is age dependent. Clinically, it has been shown in several studies that age is one of the major determinants of outcome following stroke.

Beside the unsuccessful approach of post-ischemic neuroprotection and the mentioned experimental limitations, first exciting results from a clinical trial investigating the antimicrobial prevention of stroke-associated infections, in particular pneumonia, emphasize the pivotal role of a deranged immune system for the development of post-stroke infections, for improved neurological outcome and for the reduction of delayed mortality.

Aim:

The aim of the ARGES project is the characterisation of age-dependent changes of the immune response local (brain) and systemic (white blood cells) in an animal model of mice using transcriptomics, proteomics and partially on a biochemical level. We focus on biomarkers in the peripheral blood of (stroke) animals by functional genomics to transfer knowledge gained in this approach to human samples. In these study two novel therapeutic approaches affecting the inflammatory reactions (sphingomyelin-ceramide-cycle, Von-Willebrand-Factor) will by experimentally asses (in animals).



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In detail, ARGES will

(i) analyse a divergence in the age-dependent inflammatory reaction,

(ii) determine the relevance of an interaction of the inflammatory system and the coagulation system for the triggering of the secondary insult as potential prediction markers for the onset, risk stratification and outcome,

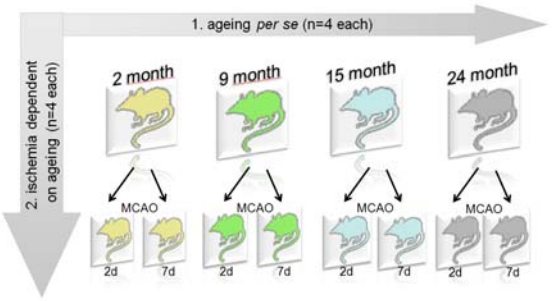
(iii) provide novel and age-adapted therapeutical and interventional strategies.

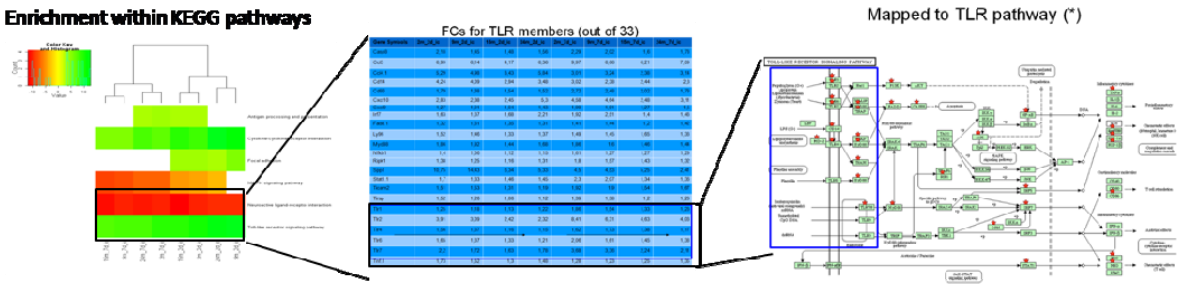
ARGES is a specific targeted research project focussing on a multidisciplinary fundamental approach (functional genomics, proteomics, biochemical experiments, animal experiments, generation of transgenic animals) to examine age-depended differences in the inflammatory response to stroke.

Results:

Aging is a major risk factor for a variety of neurobiological diseases leading to variations of transcriptional and protein expression in affected tissues. In healthy organisms the balance between pro- and anti-inflammatory reactions is a prerequisite to ensure an appropriate immune response. In the ARGES project we use an animal model of mice to gain age-related differences in the (inflammatory) responses to stroke (MCAO model) and combine multidisciplinary approaches including functional genomics, proteomics, biochemical experiments, animal experiments and patient study.

Using the whole genome chip array technology we are able to measure the gene expression of about 26,500 genetic probes in 112 brain and 20,000 genetic probes in 80 blood samples of mice. This data matrix of about 4.5 Million data points was statististical analysed to get significant regulated genes. Based on this data we identified new key regulatory pathways, affected by stroke in combination with ageing (see Workflow below).





Thereby, genes specific for microglial cells, the immune competent cells of the CNS, seem to be different regulated after stroke dependent on ageing. We found changes in gene expression responsible for antigen presentation, migration and phagozytosis. Thus, there is strong evidence that the fine balanced system of immune response is disturbed due to ageing. This disorder could lead to a second hit which could increase the neurological damage after stroke.

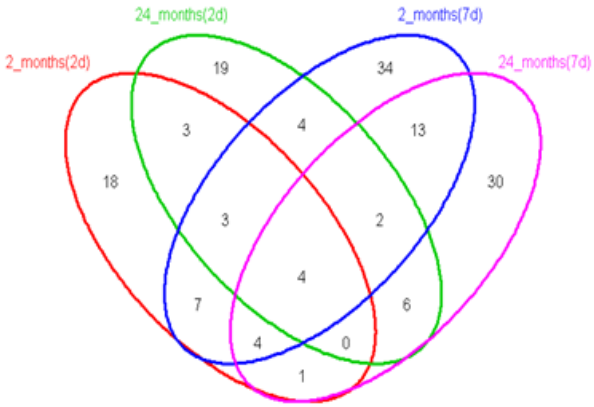
So we are able to define novel genes and pathways responsible for different recovery after stroke dependent on ageing. On the basis of these data we characterized the (inflammatory) response local in brain and systemic in white blood cells to pave the way for new therapeutical interventions.

The genes and the resulting target structures identified by expression studies in the animal model will be added to a set of candidate genes and evaluated in a parallel clinical study. Thus we hope,

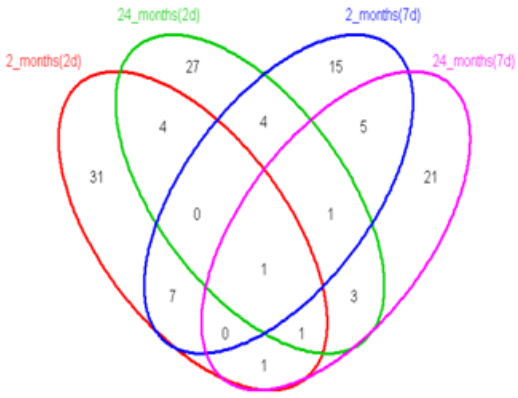
that measurement of transcriptional response in white blood cells in patients as well as in animal model allows the definition of novel surrogate markers. General we expect that these data will give a principle view on the consequences of stroke at different ages.

To identify proteins regulated by stroke in an age dependent manner two-Dimensional Differential Gel Electrophoresis (2-DIGE) in combination with high sensitive fluorescence detection was performed. This assay comprising 112 protein samples enables us to detect 702 protein spots. The data set of about 80.000 expression values was statistical analysed to select 100 protein spots for identification by mass spectroscopy. These proteins have been assigned to different signal pathways. Thereby results obtained from transcriptom analysis could be confirmed on protein level. These very promising signal cascades that are differently affected by ageing will determine our future research.

upregulated proteins (FC> 1.5) ipsi vs sham(2m,24m,2d,7d)



downregulated proteins (FC< -1.5) ipsi vs sham (2m,24m,2d,7d)



Determining the lipid composition of brains with and without MCAO, we found (i) that with respect to sphingolipids and its derivatives such as ceramides the ratio varies depending on age and (ii) that the response to MCAO with respect to formation of ceramides is also different offering therapeutical alternatives to prevent overwhelming ceramide formation resulting from ischemia using low molecular weight inhibitors.

Potential applications:

The study provides basic data on age-related changes in the transcriptome and the proteom, as well as the recovery and mortality after stroke. Provided that the main conclusion - relative independence of age of the recovery process - can be confirmed with human data later on, these results will strongly affect the therapy, the therapeutic effort and future therapeutic research directed to the treatment of elderly European stroke patients. The potential applications that might arise from this project are (i) new diagnostic options to monitor the systemic (inflammatory) response after stroke and (ii) new age adjusted therapeutic options to reduce the effects caused by the overreaching inflammatory reaction in brain and the systemic immune suppression later on after stroke

Key words: Neurobiology, Stroke, Apoplex, Aging, Inflammation, Ischemia, Brain, White Blood Cells, Transcriptomics, Proteomics, Lipidomics,

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