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Meeting Report

Targeting the immune system in disease. Highlights from the Society for Medicines Research meeting

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Summary

Immune homeostasis and surveillance are fundamental mechanisms that protect from environmental stress, infections and disease. Recent advances have shown us how dysfunction of the immune system and overt inflammation contribute to the development and worsening of major non-communicable diseases, including neurodegenerative disease, cancer, cardiovascular disease, diabetes and many others. The purpose of this Society for Medicines Research (SMR) Conference was to discuss the latest biological breakthroughs in immunology and how these discoveries are being applied to understand and target fundamental pathological mechanisms across therapeutic areas. The meeting brought together renowned experts who are making fundamental breakthroughs in disease areas such as neuroinflammation, cardiovascular disease and immuno-oncology to discuss the latest advances and outline important areas for future research. The meeting highlighted our increasing understanding of key inflammatory pathways that underlie the progressive pathology seen across a range of disease states such as rheumatoid arthritis, cardiovascular disease, oncology and Alzheimer's disease. Furthermore, we learnt how these discoveries are being translated into novel therapies that are designed to target key inflammatory pathways and modify human disease. This SMR symposium was held at the Chancellors Conference Centre, Manchester, hosted by the University of Manchester, and sponsored by Astex Pharmaceuticals, Immunocore and the Merck Group.

Key words: Alzheimer's disease – Microglia – Rheumatoid arthritis – Stroke – Cardiovascular disease – TNF-α – Cytokines – NLRP3 inflammasome – Immuno-oncology – Glioblastoma – Innate immunity – Cancer

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Anti-TNF Therapy: One of the Early Successes of Targeting the Immune System: Could We Improve on It?

Prof. Sir Marc Feldmann, University of Oxford, initiated the meeting by stressing the need for an integrated approach to therapy. Classically, most companies were looking for a single "miracle cure" to address an unmet need, whereas in today's real medical practice every major disease is treated with a combination of medicines. Prof. Feldmann treated the

delegates to a wonderful account of his seminal contributions to the field, beginning with his early 1980s concept linking aberrant HLA-DR expression and antigen presentation, presumably driven by cytokines in the induction of autoimmunity. Probing this hypothesis in the context of rheumatoid arthritis revealed that many cytokines are produced in the rheumatoid synovium, including proinflammatory cytokines interleukin-1 (IL-1) and IL-6 and anti-inflammatory IL-10. The importance of tumor necrosis factor (TNF) was revealed by analysis of cytokine regulation (1) in diseased synovium which led to the concept of a TNF-dependent cytokine cascade in rheumatoid arthritis. The delegates were shown a video of Feldmann and Maini's first proof-of-principle trial demonstrating that 10 mg/kg anti-TNF therapy yielded major improvement. Building on a subsequent randomized, placebo controlled study (2), which delivered formal proof-of-principle, a breakthrough increase in efficacy for patients not responding to current medications (including methotrexate) was achieved by combining anti-TNF with methotrexate (3).

Prof. Feldmann highlighted that anti-TNF has triggered the monoclonal antibody revolution, being the first antibody used long term for a common disease. Approximately 10 million patients have been treated worldwide with major therapeutic benefits, though there is still no cure. Since 2015, more than 20 anti-TNF biosimilars have entered the clinic with about half a dozen approved.

In the closing part of the lecture, the partial benefit seen with five different anti-TNFs (4) was contrasted with staggering marketing figures; anti-TNF is the most lucrative drug class with global sales in 2016 totaling ~USD 36 billion. Prof. Feldmann stated that early treatment of rheumatoid arthritis with anti-TNFs is now much more effective, yielding close to a cure for some patients, but this is not yet possible in routine care. In an effort to mimic this success he is now pursuing an approach that combines inhibition of stromal cells with anti-TNF and methotrexate treatment. In support of this approach, studies by a collaborator, Prof. Itoh, have shown that fibroblast-like stromal cell growth inhibition with anti-matrix metalloproteinase-14 (MMP-14) on top of anti-TNF was synergistic in mouse models of arthritis. To pursue more effective therapy and get closer to a cure, Prof. Feldmann is in the process of setting up a small company to undertake trials. His company has chosen to work with SomaLogic (Boulder, Colorado, US) who has a platform that can assay 5,000 proteins simultaneously in 60 µL of plasma, thus allowing stratification of patients in these trials. He is confident improvements in treatment are likely.

Metabolic Programming and NLRP3: Critical Determinants of Inflammation and Innate Immunity

Prof. Luke O'Neill, Trinity College Dublin, gave an exciting talk on aspects of immunometabolism. He introduced the NOD-like receptor family (NLR) protein NLRP3 (NACHT, LRR and PYD domains-containing protein 3), which is an intracellular signaling molecule that senses many pathogen-, environmental- and host-derived factors (5). Upon activation, NLRP3 binds to apoptosis-associated speck-like protein containing a CARD (ASC) which in turn interacts with the cysteine protease caspase-1, forming the complex known as the NLRP3 inflammasome. The NLRP3 inflammasome is a pivotal component underlying the induction of the proinflammatory cytokine IL-1β, which has been suggested to be a key factor in numerous diseases where inflammation is thought to play a central role such as gout, type 2 diabetes and Alzheimer's disease. Professor O'Neill highlighted the discovery of the sulfonyl urea-containing NLRP3 small-molecule inhibitor MCC-950, which has been shown to produce efficacy across a range of preclinical models (6), leading to the suggestion that this compound could potentially be beneficial against a range of conditions. NLRP3 and IL-1β activation form part of the metabolic changes which are hallmarks of the changes innate immune cells undergo during inflammatory reprogramming (7). The role of a particular metabolic phenomenon, the Warburg effect, has been recognized in the context of inflammasome activity as lipopolysaccharide (LPS)-induced signaling causes metabolic shifts in dendritic inflammatory cells from oxidative phosphorylation to aerobic glycolysis (8). The Warburg effect is also a key feature in the context of inflammation and IL-1β production. Here, Prof. O'Neill referred to a key contribution from Ridker et al. (9) that reported anti-inflammatory therapy with canakinumab (Ilaris; Novartis) and hence linked IL-1β to atherosclerosis. The final section of the talk showcased some cutting edge, unpublished work emerging from the O'Neill laboratory demonstrating how intermediates in the Krebs cycle may play key roles in modulating inflammatory responses. He showed how succinate induces a range of downstream consequences including hypoxia-inducible factor 1α (HIF-1α) and subsequently IL-1β leading to an altered inflammatory signaling. He also suggested that the conversion of citrate to itaconate, mediated by immunoresponsive gene 1 (Irg1), protects against LPS-induced inflammatory changes in mice and hence itaconate may be an anti-inflammatory agent. The underlying mechanisms of action may be via activating nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2), alkylating Kelch-like ECH-associated protein 1 (Keap1) and subsequent inhibiting interferon (IFN)-β. This expands on Kobayashi's study (10) that identified Nrf2 as an upstream regulator of cytokine production and revealed a molecular basis for an Nrf2-mediated approach to inflammation. These findings may lead to a re-framing of the Krebs cycle and its much wider function in inflammatory cellular reprogramming. The community will look forward to observing how Prof. O'Neill's studies pioneer this emerging sphere of biochemistry and the resultant impact on therapeutic approaches.

Innate Immune Activation in Alzheimer's Disease

Prof. Michael Heneka, Director of the Department of Neurodegenerative Diseases and Gerontopsychiatry at the University of Bonn, opened the session on neuroinflammation by giving a lecture about the regulation of innate immunity in the brains of people with Alzheimer's disease and how it contributes to disease progression. There is an increasing understanding that inflammation and innate immune processes contribute to the worsening of Alzheimer's disease (11, 12). At the heart of innate immunity are proinflammatory cytokines of the IL-1 family (specifically IL-1β and IL-18) that are regulated by multi-molecular protein complexes called inflammasomes. These are formed by the interaction of a cytoplasmic pattern recognition receptor (PRR), which in the case of Alzheimer's disease is often reported to be NLRP3, the adaptor protein ASC, and the proinflammatory protease caspase-1 which activates IL-1β and IL-18 (13). Several years ago Prof. Heneka first published that NLRP3 could be important in Alzheimer's disease (14). He discovered that crossing amyloid precursor protein (APP)/presenilin 1 (PS1) mutant Alzheimer's mice with NLRP3 knockout (KO) mice abolished memory deficits and reduced amyloid plaque burden (14). In the brains of APP/PS1 mice there was active caspase-1 present, whereas in the NLRP3 KO/APP/PS1 cross activation of this enzyme was inhibited. Caspase-1 activation was also observed in post-mortem brain tissue from people who had Alzheimer's disease (14). Several subsequent studies have since reported that pharmacological inhibition of the NLRP3 inflammasome in mouse models of Alzheimer's disease is protective, further supporting the emergence of NLRP3 as a potential therapeutic target (15, 16). Working closely with Prof. Eicke Latz, who had previously reported the proinflammatory effects of released ASC inflammasome specks (17, 18), Prof. Heneka investigated the potential role of extracellular specks in Alzheimer's disease. He had observed that ASC specks released into the extracellular space from microglia were quickly covered in β-amyloid (Aβ) (19). This led to the proposal that ASC specks released from microglia could seed the deposition of Aβ plaques. Prof. Heneka further illustrated this in a series of elegant experiments showing that ASC specks drove Aβ oligomerization and Aβ plaque deposition in vitro and in vivo (19). Injecting the homogenates of APP/PS1 brains into the brains of APP/PS1 mice caused deposition of Aβ plaques and this was inhibited by coinjection of an anti-ASC antibody, or when ASC KO brain lysates were injected (19). In conclusion, Prof. Heneka described that inflammation and Aβ deposition occur early and before the onset of the symptoms of Alzheimer's disease, and that activation of the NLRP3 inflammasome in microglia leads to the release of ASC inflammasome specks which seed the deposition of Aβ plaques ultimately leading to neuronal loss. This work suggests that the NLRP3 inflammasome pathway could be a viable target for new therapies to treat Alzheimer's disease.

Targeting Microglial Proliferation in Alzheimer's Disease

The second talk in the neuroinflammation session was delivered by Dr. Diego Gomez-Nicola, a Principal Research Fellow within Biological Sciences at the University of Southampton. Dr. Gomez-Nicola started his talk by highlighting the role of innate immunity in Alzheimer's disease. He used genome-wide association study (GWAS) data as examples (20) and emphasized that within the brain microglia are the main effectors of innate immunity (21). He highlighted recent work showing functional diversity in microglial phenotypes in Alzheimer's disease (22) and went on to present the hypothesis that microglial proliferation contributes to the worsening of Alzheimer's disease. Dr. Gomez-Nicola explained how microglial proliferation is regulated by the colony-stimulating factor 1 receptor (CSF-1R), and that in models of neurodegeneration there is increased microglial proliferation dependent upon CSF-1R function (23). Previous studies from his group demonstrated how CSF-1R was upregulated in the brain in Alzheimer's disease and that this correlated with increased microglia proliferation (24). Thus microglial proliferation can be targeted by blocking CSF-1R activation and the CSF-1R antagonist GW-2580 can be used to inhibit microglial proliferation or, at higher doses, stimulate their removal. Furthermore, at the same doses which inhibit microglial proliferation, GW-2580 produces positive behavioral outcomes in the APP/PS1 mouse, suggesting that they are protected by CSF-1R antagonist treatment (24). Interestingly, the protective effects of CSF-1R inhibition did not correlate with any changed in Aβ plaque burden, yet it did inhibit synapse loss (24). These data suggest that CSF-1R antagonism may be a potential treatment for Alzheimer's disease independent of any effects on amyloid plaque burden. In conclusion, Dr. Gomez-Nicola summarized the microglia as necessary transducers of the toxic effects of Aβ plaques and that their CSF-1R-dependent proliferation represents a therapeutic target. While microglia may have many beneficial effects, the take home message from Dr. Gomez-Nicola's lecture was that in an Alzheimer's disease brain, microglia have a net negative effect and that this can be targeted.

Inflammation as a Therapeutic Target in Cerebrovascular Disease

Prof. Stuart Allan (University of Manchester) provided an insightful presentation into the potential scope and progress of anti-inflammatory therapy for the treatment of stroke. In his introduction Prof. Allan outlined the massive burden stroke places on society and healthcare systems. It is the second leading cause of death worldwide and a leading cause of neurological disability. There are two major causes of the disease that are categorized according to the underlying pathology: ischemic stroke (approx. 80% of cases),

which occurs as a result of an obstruction within a blood vessel, and hemorrhagic stroke (approx. 20% of cases), which is caused by rupture of weakened blood vessels in the brain. Despite the huge unmet need, treatment options are extremely limited and mainly consist of reopening the occluded blood vessels, a therapy only effective in ischemic stroke. The lack of drugs for this indication is at least in part due to the fact that most clinical trials in stroke have failed so far, causing the industry to shy away from this area and resulting in a very limited number of new treatments going through clinical testing.

Inflammation is regarded as a major contributor to stroke pathology and has been highlighted in a variety of studies as a process that contributes to stroke risk, acute damage as well as long-term complications post insult. The innate immune response appears to play a very important role early after stroke, making the idea of targeting this part of the immune system an attractive proposition. Among the many inflammatory processes that are being triggered by innate immune cells, the IL-1 pathway has been increasingly recognized as a potential drug target. For example, work at the University of Manchester has highlighted the increased expression of IL-1α in microglia within the ischemic region of murine brain early after cerebral occlusion/reperfusion (25).

In the next part of his talk Prof. Allan outlined how, based on the strong rationale, the Manchester team investigated the potential benefit of IL-1 receptor antagonism in models of cerebral ischemia (middle cerebral artery occlusion [MCAO]) and tackled a number of critical issues that previously had not been addressed. For example, the group demonstrated that the IL-1 receptor antagonist anakinra (Kineret; Swedish Orphan Biovitrum), a drug approved for the treatment of rheumatoid arthritis, can penetrate the blood–brain barrier (BBB) and reduce infarct volume in a stroke model, when injected subcutaneously. It was also shown that in the context of comorbidity, e.g., obesity or infection, the drug was still effective. Delayed dosing of anakinra (3 hours post insult) reduced lesion volume (26), and acute treatment led to sustained protection and recovery which was still observed after 1 month, highlighting the potential benefit of this drug when used early post insult (27). Very recent data also showed that IL-1 receptor antagonism promotes neurogenesis in a rodent stroke model (28). Remarkably, the Manchester group initiated a preclinical cross-laboratory stroke trial designed to investigate the efficacy of IL-1 receptor antagonist in different research laboratories across Europe (29). This noteworthy effort, in which the protective activity of anakinra in cerebral ischemia was reproduced by several groups, further strengthened the therapeutic potential of IL-1 receptor antagonist in experimental stroke.

Having made such a strong case for IL-1 receptor antagonism in preclinical models, the Manchester group proceeded to human trials (29). Proof-of-concept data were obtained in a subarachnoid hemorrhage phase II trial that demonstrated reduced plasma inflammation. In an additional ischemic stroke phase II trial the primary endpoint (reduction of IL-6) was demonstrated. A phase III study in subarachnoid hemorrhage and a phase II trial in intracerebral hemorrhage are scheduled to start this year.

In the final part of his talk Prof. Allan outlined mechanistic studies to explore the mechanism by which IL-1 receptor antagonism provides benefit in cerebral ischemia. Targeted knockout studies of IL-1α and -β in MCAO mice led to the conclusion that both peripheral and central IL-1 play a role in stroke pathology. Given the function and expression of the IL-1 receptor it appears that further benefit in IL-1 receptor antagonist therapy could be achieved by elevating drug levels in the CNS and target processes that lead to neuronal loss and impairment of regeneration. Efforts have therefore been initiated to create liposomal formulations that could increase the delivery of drug to the CNS and provide additional benefit.

Overall, Prof. Allan made a compelling case for IL-1 as a promising target for stroke, with the positive outlook that finally, after many years, a new drug to treat one of the world's most devastating diseases may be on the horizon.

Role of the Inflammasome in Meta-Inflammation and Atherosclerosis

Prof. Eicke Latz (University of Bonn/University of Massachusetts) gave a comprehensive lecture on the link between obesity, chronic noncommunicable diseases, inflammation and reprogramming of the innate immune system.

In the first part of his talk, Prof. Latz outlined the increasing threat to global health posed by noncommunicable diseases in the context of obesity and Western-type calorically rich diets ("western diets"). These noncommunicable diseases, in particular cardiovascular disease, type 2 diabetes and obesity, now account for more than 80% of deaths worldwide (30) and are frequently linked to high cholesterol and metabolic changes induced by the consumption of fast food containing high levels of fat, glucose, cholesterol and salt. Worryingly, this trend is on the increase, and it has been predicted that the current generation of children in the U.S. will be the first generation to have a shorter lifespan than the previous. Similarly, obesity is strongly increasing in other parts of the world, for example in the urban areas of China, India and developed parts of Africa, where consumption of fast foods has become popular.

Pathologically, many of the features that contribute to noncommunicable illnesses are linked to inflammation, and western diet-induced changes in lipid or glucose levels can alter immune cell function. Chronic inflammatory processes have been detected in diseased tissue, e.g., pancreatic

islets of type 2 diabetes patients or atherosclerotic plaques, and it has been suggested that they contribute to disease progression (31). These processes involve cells of the innate immune system, and Prof. Latz's team has conducted a number of elegant studies to elucidate the mechanisms that link western diet to increased tissue inflammation (32).

As an introduction to this part of his presentation, Prof. Latz explained the role of innate immune memory in this context. It has been observed that, after an initial encounter with pathogens, the innate immune system is able to respond more vigorously to subsequent infections, a mechanism mediated by epigenetic and metabolic reprogramming that can last for prolonged periods of time. Early evidence exists that innate memory may also occur in the context of obesity and atherosclerosis (33), raising the possibility that this phenomenon could contribute to disease pathology. Dr. Latz's team chose an in vivo systems approach to evaluate the role of innate immune memory in diet-induced atherosclerosis, focused on the early stages of plaque formation. These studies were done using the $Ldlr^{-/-}$ atherosclerosis model, where LDL-receptor-deficient mice were fed a high-fat western diet for 4 weeks, followed by 4 weeks of normal chow. Transcriptome and protein analysis was carried out across different innate immune cell populations in various compartments (e.g., microglia, spleen macrophages, bone marrow myeloid precursors). Initial serum cytokine analysis revealed diet-induced transient elevation of proinflammatory cytokines that reverted to normal levels after change to normal chow. Innate immune cell responses to proinflammatory stimuli were, however, maintained beyond the 4-week western diet feeding period. More detailed analysis revealed that both splenic macrophages and bone marrow cells were functionally reprogrammed by the western diet. An increase in ratio between circulating granulocytes and lymphocytes, a phenomenon linked to severe coronary artery disease in humans, was also observed.

Prof. Latz then led the audience through a series of comprehensive transcriptomic and cellular data on granulocyte/monocyte precursors which strongly supported the notion that western diet induces transcriptional reprogramming, leaving innate immune cells in a primed activation state that promotes inflammatory responses. Additional ex vivo and in vivo experiments using LPS stimulation revealed that western diet-induced reprogramming is long-lasting and mediated through epigenetic changes.

Once it was convincingly demonstrated that western diet consumption leads to innate immune cell reprogramming in mice, additional experiments in human cells and murine knockout models were conducted to identify the mechanisms that control these cellular changes. These elaborate studies identified the NLRP3 inflammasome as the key mediator of diet-induced cell reprogramming, suggesting that inhibition of this complex may offer a therapeutic opportunity to inhibit chronic inflammation and prevent disease.

Finally, Prof. Latz closed his fascinating lecture with the comment that ultimately only better education about the dangers posed by junk food will lead to a reduction in many noncommunicable illnesses and improvements in global health.

Making and Breaking Tumor Tolerance

Prof. Andrew Mellor (Professor of Translational Immunology, University of Newcastle), gave a fascinating overview of the central role that DNA sensing to activate STING (stimulator of interferon genes)/IFN-I signaling plays in controlling local tumor immune balance and tolerance. The presentation focused on the three overlapping themes of i) chronic inflammation and immune balance, ii) cancer as a chronic inflammatory disease and iii) chronic disease comorbidities.

Inflammation was presented as the "Ying and Yang" of tissue context-specific processes; for example: "smouldering" inflammation produced by cancer and chronic infections versus "wildfire" inflammation that can be triggered by autoimmunity, allergy and transplant rejection. Central to this are "danger" signals or inflammatory insults (such as infections, tissue damage, cell death leading to DNA release, etc.) that can stimulate innate immune cells leading to production of IFN-I (e.g., via STING signaling) (34). Depending on the tissue context and local immune balance, IFN-I can drive proinflammatory and regulatory immune responses that contribute to immunity (e.g., pathogen clearance, tumor rejection), and/or suppression and tolerance (e.g., tissue healing, tumor growth) (35). An important component of the tolerogenic pathway is an immune checkpoint known as indoleamine 2,3 dioxygenase (IDO) that catabolizes tryptophan to generate kynurenines, which promote immunosuppressive regulatory T cell (Treg) activation and suppress immune effector cells. Increased kynurenine production is also associated with increased sensitivity to pain and depression. Interestingly, it has been shown in a B6 mouse model skin graft rejection that DNA from dying/apoptotic cells can suppress immunity and allow skin grafts to survive via STING/IFN-I signaling and induction of IDO. Due to this, the question was then posed, "Does DNA also protect tumors and infected cells" from the immune system?

It is well known that in most if not all people, cells acquire mutations in genes controlling proliferation and that the immune system prevents premalignant lesions from forming malignant tumors. Even so, in some instances local inflammation leads to dominance of immune tolerance in the tumor microenvironment (TME)—a hallmark of cancer at clinical presentation. Due to this, Prof. Mellor and his group set out to determine whether DNA from dying tumor cells is sensed leading to STING activation/induction of

IDO and if tumor antigenicity impacts immune balance in the TME. Using the Lewis lung carcinoma (LLC; cells do not express IDO) mouse model they demonstrated that ([34] and unpublished data) i) LLC engraftment induces rapid and sustained elevation of IDO enzyme activity by dendritic cells located in local lymph nodes; ii) DNA sensing activates STING and induces IDO expression since this response did not occur in mice lacking STING genes; iii) DNA sensing promoted LLC growth (knockout of either STING or IDO-1 significantly decreased tumor growth in vivo); iv) increased tumor antigenicity inhibits tolerogenic responses to DNA in the TME (introduction of neotumor antigens to LLC cells blocked induction of IDO enzyme activity and STING or IDO-1 knockout impeded LLC-gp100 growth in vivo); v) STING agonists (cyclic diAMP) are only effective against dermal LLC tumors by direct injection, whereas they can eliminate LLC lung tumors (produced by i.v. injection of LLC cells) when they are given either intravenously or intranasally.

Overall, the data show that elevated tumor antigenicity reduces tolerogenic barriers in the TME, DNA sensing impedes growth of highly antigenic tumors and systemic and local responses to STING agonists induce effective therapeutic responses. In addition, STING agonists have been shown to overcome immune checkpoints and promote antitumor activity by several different groups. Data from non-small cell lung cancer patients has also shown that IDO activity correlates with poor clinical responses and survival following radiotherapy, and that serum IDO activity is a prognostic marker of patient responsiveness to radiotherapy (36). Based on this data and similar results from other tumor types suggesting that IDO expression is prognostic of poor survival, several IDO inhibitors are now being actively developed in the clinic (37).

The final part of the presentation introduced the role of IDO in pain. Data were shown providing evidence for virus infection and STING agonists enhancing pain sensitivity in a mouse model of influenza by induction of IDO. This sensitivity was reduced by application of a 3-hydroxyanthranilic acid dioxygenase inhibitor (HAAO; downstream of IDO in the kynurenine pathway that converts 3-hydroxyanthranilic acid to quinolinic acid, itself an NMDA [*N*-methyl-D-aspartate] precursor) due to blockade of NMDA production and neuronal pain (38). LLC growth and STING agonists were also shown to enhance pain sensitivity in the LLC mouse model.

In summary, Prof. Mellor demonstrated that interferons are pivotal factors controlling local immune balance and that DNA may be immunogenic or tolerogenic via STING/IFN-I. In addition, DNA induces IDO, a common immune checkpoint and biomarker of clinical response in cancer, to promote tolerance (via STING/IFN-I), disrupt local tumor tolerance and enhance pain sensitivity. Finally, IDO inhibitors have the potential to break tumor tolerance leading to lower resistance to therapy, higher tumor antigenicity and improved responses to therapeutic cancer vaccines when given in combination.

Ameliorating Adverse Effects of Radiotherapy on the Normal Brain by Targeting Neuroinflammation

Prof. Anthony Chalmers (Chair of Clinical Oncology, University of Glasgow) and Prof. Kaye Williams (Chair of Experimental Therapeutics, University of Manchester) gave an excellent overview on the challenges of treating glioblastoma (GBM), an aggressive form of brain cancer with 100% mortality, and the potential role of poly(ADP-ribose) polymerase (PARP) inhibitors in both improving the tumor's response to conventional therapy and protecting normal brain tissue from neuroinflammation induced by radiotherapy.

GBM is an orphan disease with a median overall survival of around 15 months with standard first-line therapy consisting of maximal safe tumor resection, followed by concomitant chemoradiotherapy (RT plus daily temozolomide [TMZ]) and six 28-day cycles of adjuvant TMZ (39). GBM is also a highly infiltrative tumor and due to this, large volumes of the brain need to be irradiated using high doses. This appears to generate neuroinflammation in all areas of the brain, even outside of the irradiated area, leading to neurotoxicity, often with dire consequences to normal cognitive function and quality of life such as dementia (40).

It has been demonstrated in preclinical cancer models that inhibition of the DNA repair protein PARP can enhance the antitumor effect of RT (41). PARP is also thought to be involved in the pathogenesis of neuroinflammation. In addition, a stem-like population of GBM cells have upregulated DNA damage repair pathways and are resistant to RT which can be overcome with addition of PARP inhibitors (42). A major challenge with the application of such drugs in the clinical GBM setting was the notion that they could not cross the intact BBB. Even so, it is known that the BBB is grossly disrupted, and Prof. Chalmers led a Cancer Research UK-sponsored clinical study demonstrating that the PARP inhibitor olaparib (Lynparza; AstraZeneca) could penetrate GBM tumors (43), even reaching therapeutically relevant levels in tumor margins. Follow-on clinical trials (PARADIGM and PARADIGM-2) are now examining whether addition of PARP inhibitors to standard of care for GBM can improve patient survival. This data, along with preclinical data in mouse models of stroke and traumatic brain injury suggesting that PARP inhibition may provide neuroprotection (44, 45), raises the possibility that this approach may augment tumor responses to radiotherapy while protecting normal brain tissue.

The main goal of the current collaboration between Professors Chalmers and Williams is to try and address the issue of neuroinflammation through a better understanding of the biological processes triggered by RT of the brain.

A multidisciplinary research program has been set up to i) integrate noninvasive imaging, behavioral studies and ex vivo tissue analyses; ii) define temporal effects and potential mediators; iii) develop robust models to evaluate therapeutic interventions, such as PARP inhibitors with (potential) companion PD biomarkers.

Noninvasive imaging is being used on the principle that neuroinflammation is associated with increased expression of the 18 kDa translocator protein (TSPO) which is present on the mitochondria of activated microglia, astroglia and macrophages. Upregulation of TSPO can also be imaged using positron emission tomography (PET) ligands (46). Initial work was performed using mouse models of Alzheimer's which demonstrated that chronic inflammation could be tracked using the TSPO PET ligand [^{18}F]DPA-714. Building on this proof-of-concept for TSPO PET, mice were exposed to 20 Gy single fraction RT applied to right hemisphere and PET/CT scans performed. These were done prior to and once a month from 1-6 months post-RT. The studies showed that the increased neuroinflammation found at 1-month post-RT had resolved at around 6 months. Notably, baseline inflammation increased with age, and uptake of tracer indicated a global effect beyond the region of RT in both hemispheres. Preliminary ^{1}H-mass resonance spectroscopy (MRS) also showed perturbation in glutamate/glutamine ratio at late stages post-RT, potentially indicative of nerve cell damage and/or loss. Next steps in the program are to demonstrate whether addition of PARP inhibitor to RT can ameliorate neuroinflammation post-RT using TSPO PET, complementary MRS and behavioral studies.

The outcome of these preclinical and clinical studies should help to determine whether PARP inhibition can enhance tumor response while ameliorating normal tissue damage following radiotherapy in GBM, and have the potential to have significant impact on patients by extending long-term survival post-RT.

Disclosures

L.A. Dawson, S. Allan, D. Brough, J. Ritchie, P. Weber and S.P. Wren are in paid employment of their respective organizations. L.A. Dawson, J. Ritchie, P. Weber and S.P. Wren are Society for Medicines Research Committee members for which no remuneration is paid.

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