Chapter 10

Valorisation

Although (organ) transplantation is currently mostly successful, there are still complications such as transplant rejection, where the recipient's immune system targets the graft, which can lead to graft failure and removal of the organ [1,2]. Matching for HLA and ABO blood group antigens in combination with the administration of immunosuppressive drugs has drastically improved graft outcome after transplantation [3,4]. Nevertheless, there is a subset of patients who experience difficulties after (kidney) transplantation. Therefore, enhanced treatments and monitoring tools are necessary to improve graft outcome in those patients.

Cancer (or termed malignant tumour) represents a large cluster of diseases that are capable of affecting any part of the human body and is one of the leading causes of death worldwide [5,6]. Early detection of malignant tumours and the use of enhanced conventional treatments such as surgery, RT, and chemotherapy resulted in an improved patient survival. Though, not all patients with cancer benefit from existing detection and treatment strategies. Therefore, novel treatment approaches (such as immunotherapy) and assessment tools are required.

In this thesis, we aimed to improve the understanding of the functional role of B cells and their secreted antibodies in (kidney) transplantation and cancer. We focused to what extent B cells are activated to produce antibodies after exposure to foreign antigens during (kidney) transplantation, whether we can use their secreted antibodies as biomarker after treatment or transplantation, and whether we are able to influence B cells and their effector functions in the field of cancer.

The exposure to foreign antigens activates B cells

In (kidney) transplantation, the detection of foreign (donor) HLA molecules by the recipient's immune system can trigger powerful allograft responses, which may ultimately lead to graft rejection. To prevent these allograft responses, we match HLA molecules from the recipient and donor [7]. Many studies showed the importance of *HLA-DRB1* matching. However, there is limited information on the immunogenicity of the HLA-DRB1 associated HLA-DRB3 protein.

In **chapter 3**, we examined whether HLA-DRB3 antibodies are clinically relevant. We showed that kidney transplant recipients with HLA-DRB3 antibodies displayed an inferior graft outcome as compared to recipients without these antibodies. Although our data imply that HLA-DRB3 antibodies are detrimental for kidney graft outcome, these antibodies were

accompanied with a wide-ranging alloimmunization. To further investigate the exact clinical relevance of these antibodies, large multicenter studies should be performed in future.

Importantly, we demonstrated that not only the presence or absence of *HLA-DRB3* is immunogenic, but also its' allelic diversity. Furthermore, we showed using solid phase assays that HLA-DRB3 has multiple epitopes on the surface. To date, only the presence or absence of HLA alleles are taken into account for HLA matching purposes. Nevertheless, investigating 'immunogenic' epitopes (which can give rise to alloreactivity responses) rather than existing criteria may be important for accurate HLA matching [8]. At present, the antibody reactivity epitope determination can be assessed using a computer algorithm called HLA Matchmaker. This tool consists of a database with many polymorphic residues (eplets) that are important components of immunogenic HLA epitopes. Using this tool, HLA mismatch acceptability can be assessed based on an epitope based approach and consequently suitable donors can be selected [9]. A different computer algorithm called PIRCHE (predicted indirectly recognizable HLA epitopes) predicts for example the binding of processed donor-derived HLA peptides to the recipient's HLA molecules. This tool indirectly calculates the risk via the T cell response whether specific peptides potentially derived from immunogenic epitopes on the surface of HLA molecules are more prone to induce robust antibody responses [10,11]. Our data may be of value in such computer algorithms to expand the understanding of immunogenic epitopes.

In future, matching immunogenic epitopes may be a different approach to define minimal matching criteria in transplantation. The recipient and donor are matched based on the strength of the immunogenic epitopes instead of the presence of HLA alleles. Moreover, HLA-DRB1 associated molecules such as HLA-DRB3, HLA-DRB4, and HLA-DRB5 may provide added value for such matching approach.

To date, RhD antigens are not taken into account for the allocation of solid organs (kidneys). However, female kidney transplant recipients with RhD antibodies as a consequence of RhD incompatible kidney transplantation may develop HDFN in a future pregnancy.

In **chapter 2**, we observed only 1 out of 156 (0.6%) RhD- kidney transplant recipients who developed RhD antibodies after a RhD incompatible kidney transplantation. Although this is very irregular, we showed that RhD incompatible kidney transplantation can give rise to RhD antibodies. Importantly, despite the low percentage, it may be advisable to provide anti-RhD prophylaxis to female kidney transplant recipients (< 45 years) who want to have a future pregnancy. The recommendation to administer anti-RhD prophylaxis is clinically very

relevant, since it has been widely acknowledged that RhD antibodies can cause HDFN during pregnancy [12]. In case no anti-RhD prophylaxis is given to female kidney transplant recipients and they develop HDFN, various 'expensive' treatment strategies (*e.g.* exchange transfusion) must be applied directly. Besides the costs, these treatments are invasive for the fetus and newborn infant. In contrast, to prevent the development of HDFN in these kidney transplant recipients, the administration of anti-RhD prophylaxis is a relatively 'inexpensive' choice (*e.g.* €60 per 1000 IU, RheDQuin, Sanquin).

The usage of B cells and their secreted antibodies as biomarker

CKD is a significant health problem in various Western countries. For many ESRD patients, a kidney transplantation is the treatment of choice based on the gain in life expectancy and an increased quality of life [13]. A successful kidney transplantation depends mainly on the ability to discriminate between early rejection, infectious problems, or DGF. The 'golden' standard to assess kidney function is SCr, however SCr is a relatively late marker for kidney damage [14]. Currently, a fast marker to assess kidney function directly after transplantation is absent and is crucial to improve transplant outcome in case of recipients with DGF. In the search for an advanced marker, we examined the sFLC Ig normalization after kidney transplantation.

In **chapter 4**, we showed that the κ sFLC Ig normalization was faster than SCr, MDRD, and β2-M after kidney transplantation with a graft from either a living donor, DBD, or DCD. The sFLC Ig levels were not correlated with HLA antibody responses against the transplanted graft. The most important finding was that all recipients with IGF showed complete κ sFLC Ig normalization, whereas all recipients with DGF displayed incomplete κ sFLC Ig normalization within the first week after transplantation. This 'hallmark' is clinically very relevant, since we are searching for a marker to evaluate kidney function directly after transplantation. To this end, sFLC Igs may be a new marker, however it should be noted that it remains to be proven in a multicenter study whether sFLC Igs are a predictive marker for complete kidney function or graft rejection.

Although there was a moderate correlation of sFLC Igs with SCr and MDRD, the rapid decline of both the κ sFLC Ig and β2-M levels after kidney transplantation implied that these proteins reflect efficient and immediate proximal tubular function. These findings might be of value to define graft function immediately after kidney transplantation to improve overall transplant outcome.

Several (pre)clinical studies showed that fractionated RT can result in distant non-irradiated (abscopal) tumour regression. Although it has been shown that T cells are important in this sporadic radiation-induced phenomenon, these authors do not preclude that other immune mechanisms exhibit an additional role in the abscopal effect [15-18].

In **chapter 5**, we showed that fractionated RT only or combined with DC stimulation (Flt3-L administration) stimulates abscopal responses in the 67NR mouse model. Moreover, irradiation of the primary tumour resulted in a survival benefit and a delayed growth of the non-irradiated (distant) tumour. These findings have a substantial relevance for the clinic, as it is of major importance to enhance beneficial RT-induced phenomena such as the abscopal effect. Some clinical case reports have been published about abscopal responses in patients with various types of cancer. In 2015, Golden *et al.* described a clinical trial in which patients with cancer were treated with fractionated RT in combination with GM-CSF (growth factor) administration [15]. This multimodal approach resulted in more abscopal responses and an improved patient survival. These clinical studies hint towards the application of fractionated RT combined with novel immunotherapies to boost the occurrence of abscopal responses [19].

In our study, we mainly studied the humoral anti-tumour immune response against 67NR tumour cells in Balb/C mice. We demonstrated that the 67NR tumour in these mice was associated with a pre-existing antibody response. However, the total quantity of plasma antibodies and the Ig isotype composition were not affected after fractionated RT and/or DC stimulation with Flt3-L in this model. The monitoring of anti-tumour antibodies may be used as marker for (in this case) abscopal responses. Although we showed an evident anti-tumour antibody response, assessing these antibodies in the setting of RT-induced abscopal tumour regression was not invariably associated with therapeutic effects. Nevertheless, it has to be further studied whether anti-tumour antibody responses are of value to define the efficacy of novel treatment strategies to eradicate cancer.

The stimulation or inhibition of B cells and their effector functions

The main cause of morbidity and mortality after HSCT is cGVHD. Existing immunosuppressive drugs are frequently ineffective, and novel therapeutic strategies are necessary. Although T cells (donor) play a predominant role in cGVHD pathophysiology, it has been shown that B cells also contribute to cGVHD [20].

In **chapter 6**, we demonstrated that the activation and proliferation of polyclonally

stimulated human B cells was inhibited in a dose-dependent manner after administration of JAK inhibitors ruxolitinib and fedratinib. Our findings are clinically very relevant, since these results indicated that JAK inhibitors have a profound inhibitory effect on the B cell response, which may reflect a mechanism how JAK inhibitors interfere with all fundamental pathways known in cGVHD. Importantly, the treatment efficacy in patients has to be further examined in clinical trials. To this end, several multicenter phase II/III clinical trials are validating JAK inhibitor ruxolitinib in patients receiving HSCT and suffering from GVHD (official trial number NCT02396628, NCT02913261, NCT02953678).

In addition, ruxolitinib and fedratinib or other JAK inhibitors may open new ways for the treatment of various autoimmune diseases and other B cell-related diseases.

Patients suffering from CKD and MM show elevated sFLC Ig levels and often have insufficient vaccination efficacy or infectious disease respectively. We examined whether these elevated sFLC Ig levels in CKD and MM patients play a fundamental role in the modulation of immune responses in these disorders, since the precise biological functions of sFLC Igs are unknown.

In **chapter 7**, we demonstrated that the activation and proliferation of polyclonally stimulated B cells and T cells was not affected after incubation with sera from CKD and MM patients with elevated sFLC Ig levels. Similarly, purified (unconjugated) κ and λ sFLC Igs did not influence the activation and the proliferation of stimulated B cells and T cells at different physiologically relevant doses. The clinical relevance is rather limited, because we indicated that elevated sFLC Ig levels in sera from CKD and MM patients are most likely not involved in vaccination efficacy problems or infectious disease in these patients.

In conclusion, the findings described in this thesis contribute to the general understanding of B cells are their secreted antibodies in kidney transplantation and cancer. The obtained data of this thesis may improve monitoring tools, HLA matching criteria, and provide additional rationale for anti-RhD prophylaxis in kidney transplantation. Furthermore, our findings may support the realization of novel treatments for cancer and for B cell-related diseases.

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