7 Appendix

7.1 References

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7.2 Abbreviations

Acronym	Expansion
APC	Allophycocyanin
APC	Antigen presenting cell
APC-Cy7	Allophycocyanin-cychrome7
ATCC	American Type Culture Collection
BLI	Bioluminescence imaging
BMT	Bone marrow transplantation
BSA	Bovine serum albumin
CBC	Complete blood counts
CCD	Charge coupled device
CCR	CC chemokine receptor
CFSE	Carboxyfluorescein diacetate succinimidyl ester
Cr	Chromium
CT	Computed tomography
CTL	Cytotoxic T lymphocyte
DAPI	Diamidino phenylindole dihydrochloride
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
EAE	Experimental autoimmune encephalitis
EDTA	Ethylenediaminetetraacetic acid
EGFP	Enhanced green fluorescent protein
FACS	Fluorescence activated cell sorting
FcR	Fc receptor
FCS	Fetal calf serum
FITC	Fluorescein isothiocyanate
FSC	Forward scatter
GVHD	Graft-versus-host disease
GVT	Graft-versus-tumor activity
HBSS	Hank's buffered saline solution
HEPES	Hydroxyethylpiperazine ethanesulfonic acid
HLA	Human leukocyte antigen
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
HSV1-TK	Herpes simplex virus 1 thymidine kinase

ICC	Intracellular cytokine
IEL	Intraepithelial lymphocytes
IFN	Interferon
Ip	Intraperitoneal
IPS	Idiopathic pneumonia syndrome
Iv	Intravenous
LPL	Lamina propria lymphocytes
LPS	Lipopolysaccharide
LUC	Firefly luciferase
MAB	Monoclonal antibody
MACS	Magnetic cell sorting
mHAg	Minor histocompatibility antigen
MHC	Major histocompatibility complex
MLN	Mesenteric lymph node
MLR	Mixed lymphocyte reaction
MoMLV	Moloney murine leukemia virus
mRNA	Messenger ribonucleic acid
NK cell	Natural killer cell
PBS	Phosphate buffered saline
PET	Positron emission tomography
PLN	Peripheral lymph node
PNAd	Peripheral node addressin
ROI	Region of interest
R-PE	R-phycoerythrin
RPM	Rotations per minute
RPMI	Roswell Park Memorial Institute
SEM	Standard error of the mean
SSC	Sideward scatter
TCD	T cell-depleted
TCR	T cell receptor
WT	Wild type

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7.5 Zusammenfassung auf Deutsch

Die allogene hämatopoetische Stammzelltransplantation (HSCT) ist eine bedeutende Therapieform für eine Vielzahl hämatologischer Malignome, einige solide Tumoren und bestimmte erbliche, nicht-maligne Erkrankungen des hämatopoetischen Systems. Die bedeutendste Komplikation einer allogenen hämatopoetischen Stammzelltransplantation ist die Graft-versus-host disease (GVHD), welche in 25-70% aller HLA-identischen HSCTs und in mehr als 70% aller HSCTs mit HLA-Unterschieden auftreten kann. Die GVHD wird ausgelöst von im Stammzelltransplantat enthaltenen alloreaktiven Spender T-Zellen, was sich besonders darin zeigt, dass Depletion dieser T-Zellen der effektivste Weg ist, um GVHD zu verhindern. Alloreaktive Spender T-Lymphozyten sind jedoch auch verantwortlich für den so genannten Graft-versus-tumor effect (GVT), der zu einer Verminderung der Rezidivhäufigkeit nach HSCT führt und ein große Rolle für den Gesamterfolg einer Stammzelltransplantation spielt. Die Verminderung von GVHD bei erhaltenem GVT ist daher ein vordringliches therapeutisches Ziel. Neue Studien konnten zeigen, dass die spezifische Blockade der Einwanderung von alloreaktiven T-Zellen in GVHD Zielorgane (das sind hauptsächlich Darm, Leber, Haut und Lunge) eine Möglichkeit ist, um dieses Ziel zu erreichen. Das Wanderungsverhalten von T-Zellen wird bestimmt durch die Expression von drei Gruppen von Zelloberflächenmolekülen und ihren Liganden und Rezeptoren auf dem Endothel: Selektine, Chemokin Rezeptoren und Integrine. Insbesondere Chemokin Rezeptoren scheinen eine besondere Rolle für die organ-spezifische Migration von T-Zellen während GVHD zu spielen. CC Chemokin Rezeptor 2 (CCR2) könnte eines der hierfür relevanten Moleküle sein, wie *in vitro* Studien und Studien in einigen Modellen von anderen T-Zell-mediierten Erkrankungen zeigen konnten.

In der vorliegenden Arbeit wurde anhand von Mausmodellen untersucht, welche Rolle die Expression von CCR2 auf alloreaktiven $CD8^+$ T-Lymphozyten bei der Entwicklung von GVHD nach allogener HSCT spielt. Es konnte gezeigt werden, dass $CD8^+$ T- Lymphozyten von CCR2-Knockout ($CCR2^{-/-}$) Mäusen weniger GVHD induzieren als $CD8^+$ T-Lymphozyten von Wild-Typ Mäusen des gleichen Mäusestamms. Dies hing zusammen mit einer verminderten Entwicklung von GVHD in Darm und Leber, wie anhand von histopathologischen Untersuchungen gezeigt werden konnte. Die Entwicklung von GVHD in der Haut und dem Thymus und die Immunrekonstitution waren jedoch durch Abwesenheit von CCR2 nicht beeinflusst, so dass auf eine organ-spezifische Rolle von CCR2 geschlossen werden muss. Dies bestätigte sich in einem Assay zur Untersuchung des Wanderungsverhaltens von Spender $CD8^+$ T-Lymphozyten nach HSCT, wo gezeigt werden konnte, dass $CCR2^{-/-}$ $CD8^+$ T-Lymphozyten

weniger in Darm und Leber einwandern als Wild-Typ T-Zellen, die Einwanderung in sekundäre Lymphorgane aber nicht beeinflusst war. Weitere Untersuchungen schlossen andere intrinsische Defekte (Proliferation, Aktivierung, Zytokin-Produktion, Zytotoxizität) bei $CCR2^{-/-}$ $CD8^{+}$ T-Lymphoyzten aus, so dass der einzige Grund für die beobachteten Unterschiede in GVHD Morbidität und Mortalität in dem beschriebenen Migrationsdefekt zu sehen ist.

Weiters wurde festgestellt, dass $CCR2^{-/-}$ $CD8^{+}$ T-Zellen einen intakten GVT Effekt gegen zwei verschiedene hämatopoetische Tumoren (P815 und A20) besitzen.

Zusammenfassend lässt sich feststellen, dass CCR2 die Einwanderung von alloreaktiven Spender $CD8^{+}$ T-Zellen in den Darm und die Leber nach allogener HSCT kontrolliert und die Blockade von CCR2 eine neue Möglichkeit darstellen könnte, um GVHD zu vermindern ohne den bedeutenden GVT Effekt zu beeinflussen.

7.6 Erklärung

Ich, Theis Terwey, erkläre, dass die vorgelegte Dissertationsschrift mit dem Thema „CC chemokine receptor 2 is relevant for CD8-induced graft-versus-host disease but not for graft-versus-tumor activity“ von mir selbst und ohne die unzulässige Hilfe Dritter verfasst wurde und auch in Teilen keine Kopie anderer Arbeiten darstellt und die benutzten Hilfsmittel sowie die Literatur vollständig angegeben wurden.

Theis Terwey, Berlin, den 02. Februar 2006