Abschlussbericht Teilprojekt 10.2.2

Projekttitel: Therapy of acute and chronic hepatitis C virus infection: Immunological mechanisms of viral clearance

Projektleiter: PD Dr. med. Heiner Wedemeyer, PD Dr. T. Greten, Prof. Michael P. Manns
Medizinische Hochschule Hannover
Zentrum für Innere Medizin
Carl-Neuberg-Str. 1
30625 Hannover

Telefon: +49 (0) 511 / 532-6814

Fax: +49 (0) 511 / 532-6820

E-Mail: wedemeyer.heiner@mh-hannover.de

Berichtszeitraum: 01.02.2005 – 31.01.2007

I. Kurze Darstellung

1. Aufgabenstellung

Ziel des Projektes war die Analyse verschiedener Komponenten zellulärer Immunantworten bei Patienten mit akuter und chronischer Hepatitis C. Die Experimente der 2. Förderperiode haben auf den Ergebnissen der 1. Förderperiode aufgebaut.

2. Voraussetzungen

Das Projekt wurde primär in der AG zelluläre Immunologie (PD Dr. H. Wedemeyer, Medizinische Hochschule Hannover) durchgeführt. Die Infrastruktur zur Durchführung der Experimente war etabliert. Hep-Net hat das Projekt mit einer BAT 5b-Stelle und Sachmitteln unterstützt. Essentiell war die enge Kooperation mit dem Hep-Net Study-House, hier im Besonderen die Studie zur akuten Hepatitis C. In dieser Studie wurden Blutproben gewonnen, die dann im Projekt 10.2.2 untersucht wurden. Es bestand ein enger methodische und inhaltlicher Austausch mit den anderen Projektes des Bereiches 10.2

3. Planung und Ablauf des Vorhabens

Planung und Ablauf des Vorhabens sind detailliert im initialen Antrag sowie in den Zwischenberichten beschrieben worden.
Es wurden folgende Aspekte zellulärer Immunantworten bei Patienten mit akuter und chronischer Hepatitis C im Kontext antiviraler Therapien mit Interferon untersucht:

- Bedeutung myeloider dendritischer Zellen
- Natürliche Killerzellen
- HCV-spezifische T-Zellantworten
    - Induktion HCV-spezifischer T-Zellen nach Nadelstichverletzung
    - Zytotoxische CD4+ T-Zellen bei viralen Hepatitiden
    - HCV-spezifische T-Zellen bei Patienten mit akuter Hepatitis C
    - HCV-spezifische T-Zellen bei Patienten mit akuter Hepatitis C im Verlauf einer Interferontherapie
    - HCV-spezifische T-Zellen bei nach Langzeit-Ausheilung einer akuten Hepatitis C

Die Ergebnisse sind auf zahlreichen wissenschaftlichen Tagungen vorgestellt worden und wurden in Fachzeitschriften publiziert.

4. wissenschaftlicher und technischer Stand

Es wurden methodisch hochaktuelle immunologische und molekularbiologische Techniken angewendet, die entweder bereits in der Abteilung für Gastroenterologie, Hepatologie und Endokrinologie der medizinischen Hochschule Hannover etabliert etabliert waren oder für das Projekt etabliert wurden. Die notwendigen Substanzen (Antikörper, Peptide, Tetramere, molekularbiologische Reagenzien) wurden kommerziell erworben. Schutzrechte bestehen nicht.

1. Angabe der verwendeten Fachliteratur sowie der benutzten Informations- und Dokumentationsdienste

Literatursuche wurde mittels Medline/PubMed durchgeführt. Bei der verwendeten Fachliteratur handelte es sich um Zeitschriften der Universitätsbibliotheken der Medizinischen Hochschule Hannover.

5. Zusammenarbeit mit anderen Stellen

Alle weiteren Projekte des Bereiches 10.2 (insbesondere Projekt 10.2.1 und 10.2.3), das Hep-Net Start-up Fund Projekt 15.1 sowie das Hep-net Study House.

II. Eingehende Darstellung

des erzielten Ergebnisses

Die Darstellung der Ergebnisse erfolgt wie bereits im Abschlussbericht zur 1. Förderperiode auf Englisch, da internationale Mitarbeiter im Labor an der Generierung der Ergebnisse entscheidenden Anteil hatten. Diese Mitarbeiter sprechen kein Deutsch. Auf Anfrage können deutsche Zusammenfassungen gerne nachgereicht werden.

1. Dendritic cells

Detailed results have been described in the final report for the 1st funding period. Experiments have been extended Results of this project have been accepted for publication in the Journal of Viral Hepatitis (epub dec 9 2007). The abstract of this article is quoted here:
Dendritic cells (DCs) play a central role in antiviral immunity. Conflicting data on DC function have been reported for hepatitis C virus (HCV) infection. In addition to antigen presentation and cytokine secretion, a subset of human DCs displays direct cytotoxic activity. It has been suggested that measles virus and human immunodeficiency virus (HIV) may enhance cytotoxicity of DCs potentially leading to apoptosis of activated T cells and subsequent down-regulation of antiviral immune responses. We demonstrate that CD1c-positive myeloid DCs, but not BDCA-4-positive plasmacytoid DCs, are able to kill different target cells mainly via tumour necrosis factor-related apoptosis-inducing ligand. The ability of CD1c+ DCs to lyze target cells was found to be completely impaired in patients with chronic hepatitis C (10 chronic HCV patients vs 10 healthy controls; $P < 0.001$) but not in patients with primary biliary cirrhosis. Successful antiviral therapy of chronic hepatitis C rescued the cytotoxicity of DCs. Myeloid DCs of HCV patients and healthy controls had a similar phenotype and endocytotic activity, however, the frequency of mDCs in the peripheral blood was lower ($P = 0.004$) and the allostimulatory function was weaker ($P < 0.001$) in chronic hepatitis C. Thus, in contrast to HIV and measles virus studies on monocyte-derived DCs, freshly isolated myeloid DCs of patients with hepatitis C do not show an increased but a completely abolished cytotoxic activity. The impaired DC cytotoxicity could represent a novel mechanism for the increased prevalence of autoimmunity in HCV infection.

2. Natural Killer cells

The studies on this part of the project are still ongoing. We have now extended the work on direct ex vivo analysis of patients receiving treatment for acute hepatitis C.

a.) TRAIL expression on NK cell subsets in response to interferon alpha
Type I interferons have various effects on innate and adaptive immune responses. NK cells have been shown to be functionally impaired in chronic HBV and HCV infections. NK cell subsets with specific functional profiles have been characterized and are associated with different expression levels of CD56 (CD56-dim high cytotoxic activity; CD56-bright immunoregulatory cells) and NK-CD56-dim cells are reduced in frequency in chronic HCV. Gene-array studies have shown that tumor-necrosis-factor-related-apoptosis-inducing-ligand (TRAIL) is upregulated in various cells lines and PBMC after stimulation with interferon alpha. The aim of this subproject was to investigate TRAIL-expression on NK cell subsets in patients with chronic HBV and HCV infections and in healthy controls upon in vitro stimulation with type-I-interferons.
Methods:
TRAIL-expression on T cells, NK-bright, NK dim and NKT cells was investigate after 6h and 24h in vitro stimulation with recombinant Interferon alfa-2b, PEG-Interferon alfa-2b (12kDa) and PEG-Interferon alfa-2a (40kDa) by flow cytometry.
Results:
Optimal concentrations of all three interferons induced highest TRAIL-expression levels on NK-CD56-bright cells (>90% TRAIL-positive) while NK-CD56-dim and NKT cells showed lower TRAIL expression (42-60% and 7-19% after 6h) with no significant differences between the different groups of patients. Titration experiments confirmed the weaker stimulatory

activity of PEG-IFNa-2a in vitro as compared to pegIFNa-2b and IFNa-2b in terms of TRAIL-upregulation. Cells from patients with chronic HCV showed a significantly higher TRAIL-expression on NK-CD56-bright but not non NK-CD56-dim and NKT cells as compared to healthy controls and HBV patients ($p<0.001$ and $p=0.02$, respectively). Subsequently the specific IFN-effect on TRAIL-expression (expression IFN-expression medium control) was significantly higher in healthy controls than in HBV and HCV patients on NK-CD56-bright cells (70%,39% and 22%). Patients who on showed a sustained virological response to IFN-based treatment tended to show higher IFN-induced TRAIL-expression on NK-CD56-dim cells as compared to patients with subsequent virological nonresponse.
Conclusion:
This study showed that NK cell subsets show different TRAIL-expression patterns in response to type I interferons. The significance in differences in TRAIL-expression between HCV patients, HBV patients and healthy subjects and the impact on treatment response requires future investigation.

This part was presented at the European Liver Meeting April 2007 in Bareclona, Spain

b.) Comprehensive phenotypic analysis of NK cells in acute hepatitis C virus infection
Background & Aims: NK-cells have been shown to play an important role in controlling Hepatitis C Virus infection. Changes in NK-cell frequency, NK-cell function and expression level of various killing inhibitory receptors as well natural killer cell receptor have been described in chronic hepatitis C patients. However, phenotypic patterns of NK-cells have not been systematically investigated in acute hepatitis and the effects of IFN-alpha on NK-cells are largely unknown.
Methods: 12 patients with acute HCV infection (mean age 37 years, range 20-56, 8 male, 4 female) were investigated. ALT levels ranged between 55 and 2000 U/l (mean 845 U/l). All patients were treated with PEG-IFN-alpha-2b for 24 weeks. 11 patients were HCV-RNA negative at follow up and one patient relapsed. A comprehensive phenotypic NK-cell characterization was performed by a multiparametric nine-colour flowcytometry assay studying intracellular Perforin and GranzymeB expression as well as 28 surface markers.
Results: Acute hepatitis C patients showed significantly elevated expression levels for NKp46, NKp30, LFA-1, TRAIL and CD57 on NK-CD56bright cells and for 2B4 and NKG2D on all NK-cell subsets as compared to healthy controls. In contrast none of the other markers studied including CD94, CD48, NKG2A, CD81, NKG2C, CD226, CD2, and CD7 differed on NK-cells of HCV-patients versus uninfected individuals. Molecules associated with cytotoxicity (Perforin, GranzymeB) tended to be higher expressed in acute hepatitis C. Peak ALT levels correlated positively with CD57, granzyme B, TRAIL, CD48, NKG2A, and CD25 and inversely with CD7, CD226, NKp46, CD81, CD57 and 2B4.
While TRAIL and GranzymeB increased on NK-CD56-bright cells early during treatment and declined after treatment, CD94, NKG2A, NKp30 and LFA-1 increased rather late during therapy after viral clearance. In contrast 2B4, CD2 and CD81 decreased early during treatment on all NK-cell subsets. Perforin and GranzymeB expression increased during treatment.
Conclusion: NK-cells show several phenotypic changes in acute hepatitis C virus infection which are linked to activation and cytotoxic activity. Our findings differ to recent data on chronic hepatitis C. Most killing inhibitory receptors were not altered but NKG2A expression may be increased by interferon alpha therapy. However, no clear NK-cell pattern could be linked to virological response to IFN treatment.

3. HCV-specific T cells

a. Induction of hepatitis C virus specific T cells by needle stick injury in the absence of HCV-viremia

Detailed results have been described in the final report for the 1st funding period. Experiments have been extended Results of this project have been accepted for publication

in the European Journal of Clinical Investigation (January 2007). The abstract of this article is quoted here:

BACKGROUND: The risk of hepatitis C virus (HCV) infection after occupational exposure is low with seroconversion rates between 0 and 5%. However, factors associated with natural resistance against HCV after needle stick injury are poorly defined. HCV-specific T-cell responses have been described in cross-sectional studies of exposed HCV-seronegative individuals. MATERIALS AND METHODS: In this study, we prospectively followed 10 healthcare professionals who experienced an injury with an HCV-contaminated needle. Blood samples were taken on the day or the day after the event and at different time points during follow-up for up to 32 months. HCV-specific T-cell responses were investigated directly ex vivo and in T-cell lines. RESULTS: None of the individuals became positive for HCV-RNA in serum tested with the highly sensitive transcription-mediated amplification (TMA)-assay or in peripheral blood mononuclear cells (PBMC). All of them remained anti-HCV negative throughout follow-up. At the time of injury, HCV-specific CD4+ T-cell responses were already detectable in two individuals and became detectable thereafter in three additional persons. Transient HCV-specific CD8+ T-cell responses developed in two HLA-A2 positive patients, which became negative until the most recent follow-up after 5 and 17 months, respectively. CONCLUSION: We demonstrate the development of HCV-specific T cells in HCV-exposed individuals after needle stick injury indicating subinfectious exposure to HCV. T-cell immunity against HCV may contribute to the low prevalence of HCV in medical healthcare professionals in Western countries.

b. Cytotoxic CD4+ T cells in viral hepatitis

Detailed results have been described in the final report for the 1st funding period. Experiments have been extended Results of this project have been accepted for publication in the Journal of Viral Hepatitis (August 2006). The abstract of this article is quoted here:

CD4+ T cells are thought to contribute to antiviral immune responses by secretion of cytokines thereby providing help to CD8+ T and B cells. However, perforin-positive cytotoxic CD4+ T cells have been described in human immunodeficiency virus-positive patients suggesting a role not only of CD8+ but also of CD4+ T cells for killing virus-infected cells. We investigated 76 patients with viral hepatitis [15 hepatitis B virus (HBV), 22 HBV/hepatitis D virus and 17 hepatitis C virus (HCV)] for cytotoxic CD4+ T cells. The frequency of perforin-positive CD4+ T cells in viral hepatitis was highly variable ranging from < 1% to more than 25%. Perforin-positive CD4+ T cells displayed the phenotype of terminally differentiated effector cells (CD28-, CD27-). The highest frequencies of CD4+ cytotoxic T lymphocytes (CTLs) were found in patients with delta hepatitis ($P = 0.04$ vs HBV and HCV patients), and the presence of CD4+ CTLs was associated with elevated aspartate aminotransferase levels ($P = 0.01$) and decreased platelet counts ($P = 0.03$). Perforin-positive CD4+ T cells decreased in two individuals during spontaneous clearance of acute hepatitis C. Significant associations were found between the frequency of perforin-expressing CD4+ cells and age ($P = 0.04$), perforin-positive CD8+ cells ($P < 0.001$) and perforin-positive CD4-/CD8- lymphoid cells ($P = 0.002$). Differentiated CD27- effector CD4+ CTLs can be detected in patients with viral hepatitis. In particular in patients with more advanced liver disease, the accumulation of perforin-positive T cells with age could be one correlate for the more severe course of viral hepatitis in elderly individuals.

c. HCV-specific T cells in patients with acute hepatitis C

First results have been described in the final report for the 1st funding period. Experiments have been extended and final results of this project have been accepted for publication in Antiviral Therapy (June 2007), Virology J (June 2007) and Vaccine (September 2007). The abstracts of these articles are quoted here:

Wiegand et al.: Fate and function of peripheral HCV-specific T-cells during peginterferon alpha-2b therapy of acute hepatitis C. Antiviral Therapy 2007, 12: 303-316
BACKGROUND: Strong hepatitis C virus (HCV)-specific T-cell responses are associated with spontaneous clearance of acute hepatitis C. However, recent studies described a decline in HCV-specific CD8+ T-cells during interferon treatment, suggesting that the success of acute HCV therapy might be independent of adaptive immunity. METHODS: T-cell responses of eight human leukocyte antigen (HLA)-A2-positive, acutely infected patients treated with peginterferon-alpha2b were studied by ELISPOT and proliferation assays and flow cytometry analysis using HCV-specific tetramers. RESULTS: HCV-specific T-cells predominately declined during therapy. However, diverse patterns of CD4+ and CD8+ T-cell kinetics were observed. In patients with sustained virological response chemokine receptor 3 (CXCR-3) expression of HCV-specific CD8+ T-cells was upregulated, indicating homing to the liver. Low levels of T-cells remained detectable throughout treatment and follow up. In contrast, T-cells of a relapse patient did not upregulate CXCR-3 but displayed a higher staining for annexin-V, followed by a complete loss of peripheral virus-specific CD8+ T-cells by week 12. CONCLUSIONS: Kinetics of HCV-specific T-cell responses are heterogeneous in interferon-treated patients with acute hepatitis C. The decline of T-cells might be a consequence of both apoptosis and homing. The balance between cell death and regulation of chemokine receptors might lead to different long-term outcomes.

Schlaphoff et al., Functional and phenotypic characterization of peptide-vaccine-induced HCV-specific CD8+ T cells in healthy individuals and chronic hepatitis C patients. Vaccine 2007; 25: 6793-6806.
Only very limited information on phenotype and function of vaccine-induced CD8+ T cells is available for humans. We investigated hepatitis C virus-specific CD8+ T cells after vaccination with the HCV peptide-vaccine IC41 which includes 5 MHC-class I and 3 MHC class-II-restricted epitopes. In healthy subjects, IC41 induced both HCV-specific central memory as well as effector CD8+ T cells which rapidly expanded upon antigen exposure in vitro. IFNgamma production was dependent on formulation of the synthetic peptides with the adjuvant poly-l-arginine. In chronic HCV patients, the frequency of HCV-specific CD8+ T cells increased after vaccination with a decline of CD45RA-positive effector memory cells in some but not all patients. Thus, this study suggests that HCV-specific memory cells can be induced by peptide vaccination and that a reversion of functional impaired phenotypes by therapeutic vaccination is possible in chronic HCV infection.

Meyer MF et al.. Clearance of low levels of HCV viremia in the absence of a strong adaptive immune response. Virology J 2007; 4: 58
Spontaneous clearance of hepatitis C virus (HCV) has frequently been associated with the presence of HCV-specific cellular immunity. However, there had been also reports in chimpanzees demonstrating clearance of HCV-viremia in the absence of significant levels of detectable HCV-specific cellular immune responses. We here report seven asymptomatic acute hepatitis C cases with peak HCV-RNA levels between 300 and 100,000 copies/ml who all cleared HCV-RNA spontaneously. Patients were identified by a systematic screening of 1176 consecutive new incoming offenders in a German young offender institution. Four of the seven patients never developed anti-HCV antibodies and had normal ALT levels throughout follow-up. Transient weak HCV-specific CD4+ T cell responses were detectable in five individuals which did not differ in strength and breadth from age- and sex-matched patients with chronic hepatitis C and long-term recovered patients. In contrast, HCV-specific MHC-class-I-tetramer-positive cells were found in 3 of 4 HLA-A2-positive patients. Thus, these cases highlight that clearance of low levels of HCV viremia is possible in the absence of a strong adaptive immune response which might explain the low seroconversion rate after occupational exposure to HCV.

2. des voraussichtlichen Nutzens, insbesondere der Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplanes

Die Ergebnisse zeigen grundlegende neue Erkenntnisse zur Regulation von Immunantworten bei der Hepatitis C durch antivirale Therapien auf. Diese werden bei der Entwicklung von neuen Immuntherapien Berücksichtigung finden. Die Ergebnisse sind auf wissenschaftlichen Kongressen und in hochrangigen wissenschaftlichen Journalen veröffentlicht und damit der wissenschaftlichen Öffentlichkeit zugänglich gemacht worden. Insbesondere werde die Daten für das direkte Management von Patienten mit akuter Hepatitis C, die mit Interferon behandelt werden, eine große Relevanz haben.

3. des während der Durchführung des Vorhabens dem ZE bekannt gewordenen Fortschritts auf dem Gebiet bei anderen Stellen

Mehrere andere Arbeitsgruppen weltweit beschäftigen sich mit ähnlichen Projekten. Insbesondere die Frage, ob zelluläre Immunantworten während einer IFN Therapie der akuten Hepatitis ansteigen oder abfallen wird sehr kontrovers diksutiert. Unsere Arbeit konnte in diesem Zusammenhang die Daten von Rahman et al. bestätigen und diese kontroverse Frage klären (Wiegand, Antiviral Therapy 2007). Die Arbeiten zu NK-Zellen und TRAIL sind ebenfalls von hoher Relevanz. Im März 2007 ist eine Arbeit aus eine englischen Arbeitsgruppe publiziert worden, die die Bedeutung von TRAIL auf NK-Zellen für die Hepatitis B aufzeigt. Unsere Daten zeigen nun erstmals ähnliche Ergebnisse für die Hepatitis C.

4. der erfolgten und geplanten Veröffentlichung des Ergebnisses

14. Ciesek S, Helfritz F, Lehmann U, Becker T, Strassburg CP, Neipp M., Ciner A, Fytili P, Tillmann HL, Manns MP, **Wedemeyer H**. Persistence of occult hepatitis B after explantation of the primarily infected liver. J Infect Dis 2007; *in press*.

15. Ciesek S, Liermann H, Hadem J, Greten T, Tillmann HL, Cornberg M, Aslan N, Manns MP, **Wedemeyer H**. Impaired TRAIL-dependent cytotoxic activity of CD1c-positive dendritic cells in chronic hepatitis C virus infection. J Viral Hepatitis 2008, *online Dec 09 2007.*

16. Deterding K, Suneetha PV, Schlaphoff V, Hadem J, Metzler F, Bahr MJ, Manns MP, Cornberg M, **Wedemeyer H**. Clearance of chronic HCV infection during acute delta hepatitis. Infection 2008, *online Dec14 2007.*

17. Kubitschke A, Bader C, Tillmann HL, Manns MP, Kuhn S, **Wedemeyer H**. Verletzungen mit Hepatitis C Virus (HCV)-kontaminierten Nadeln bei medizinischem Personal: Wie häufig ist eine Serokonversion wirklich? DER INTERNIST 2007; 48: 1165-72.

18. Schlaphoff V, Klade CS, Jilma B, Jelovcan SB, Cornberg M, Tauber E, Manns MP, **Wedemeyer H** and the IC41 study group. Functional and phenotypic characterization of peptide-vaccine-induced HCV-specific CD8+ T cells in healthy individuals and chronic hepatitis C patients. Vaccine 2007; 25: 6793-6806.

19. Fytili P, Tiemann C, Wang C, Schulz S, Schaffer S, Manns MP, **Wedemeyer H**. Frequency of very low HCV viremia detected by a highly sensitive HCV-RNA assay. Clin Virol 2007; 39: 308-11. *(IF 2.6)*

20. Potthoff A, Deterding K, Trautwein C, Rifai K, Manns MP, **Wedemeyer H**. Sustained HCV-RNA response and HBs-seroconversion after individualized antiviral therapy with pegylated interferon alpha plus ribavirin and active vaccination in an HCV/HBV-coinfected patient. Eur J Gastro Hepatol 2007; 19: 906-9.

21. Meyer MF, Lehmann M, Wiegand J, Cornberg M, Klade C, Manns MP, **Wedemeyer H**. Clearance of low levels of HCV viremia in the absence of a strong adaptive immune response. Virology J 2007; 4: 58 *(IF 1.9)*

22. Wiegand J, Cornberg M, Aslan N, Sarrazin C, Kubitschke A, Buggisch P, Ciner A, Jaeckel E, Manns MP, **Wedemeyer H**. Fate and function of peripheral HCV-specific T-cells

during peginterferon alpha-2b therapy of acute hepatitis C. Antiviral Therapy 2007, 12: 303-316. (IF 5.3)

23. Kubitschke A, Bahr MJ, Aslan N, Bader C, Tillmann HL, Sarrazin C, Greten T, Wiegand J, Manns MP, **Wedemeyer H**. Induction of hepatitis C virus (HCV)-specific T cells by needle stick injury in the absence of HCV-viremia. Eur J Clin Invest 2007; 37:54-64.

24. Deterding K, Tegtmeyer B, Cornberg M, Hadem J, Potthoff A, Böker KHW, Tillmann HL, Manns MP, **Wedemeyer H**. Hepatitis A virus infection suppresses hepatitis C virus replication and may lead to clearance of HCV. J Hepatol 2006; 45: 770-78. (IF 4.9)

25. Aslan N, Yurdaydin C, Wiegand J, Tillmann HL, Kaiser T, Kulmann B, Bozkaya H, Meyer M, Bozdayi AM, Manns MP, **Wedemeyer H**. Cytotoxic CD4-positive T cells in viral hepatitis. J Viral Hepatitis, 2006; 13: 505-514. (IF 2.6)

26. Potthoff A, Sarhaddar J, Wiegand J, Lichtinghagen R, Sarrazin C, Ciner A, Hadem J, Trautwein C, Manns MP, **Wedemeyer H**. Spontaneous resolution of chronic hepatitis C virus infection after antiviral treatment and relapse. Hepatol Res 2005; 31:18-23. (IF 1.5)

27. Wiegand J, Jäckel E, Cornberg M, Hinrichsen H, Dietrich M, Kröger J, Fritsch WP, Kubitschke A, Aslan N, Tillmann HL, Manns MP, **Wedemeyer H**. Long-term follow up after successful interferon therapy of acute hepatitis C virus infection. Hepatology 2004; 40:98-107. (IF 9.8)

III. Erfolgskontrollbericht

1. den Beitrag des Ergebnisses zu den förderpolitischen Zielen, z.B. des Förderprogrammes –(ggf. unter Angabe des Schwerpunkts)

Die Förderung von einzelnen Grundlagenprojekten im Bereich Immunologie hatte zum Ziel, Patienten-orientierte Forschung zu fördern und insbesondere auf eine Vernetzung mit klinischen Projekten zu achten. Diese Ziele sind in vorliegendem Projekt optimal umgesetzt worden. Alle Projekte sind direkt von Patientenstudien abhängig gewesen, insbesondere die Hep-Net Kooperation im Bereich der akuten Hepatitis C haben eine weltweit einmalige Infrastruktur geschaffen. Dadurch sind Ergebnisse generiert worden, die weltweit große Beachtung gefunden haben.

2. das wissenschaftlich-technische Ergebnis des Vorhabens, die erreichten Nebenergebnisse und die gesammelten wesentlichen Erfahrungen

Die Ergebnisse sind im vorhergehenden Abschnitt ausführlich dargestellt worden. Methodisch-technische Aspekte sind ebenfalls dargestellt. Insbesondere die Konsequenzen für andere Projekte im Hep-Net sind dabei durch häufigen Austausch zwischen den Projektleitern gut kommuniziert worden.

3. die Fortschreibung des Verwertungsplanes

-Erfindungen/Schutzrechtsanmeldungen:

keine

-wirtschaftliche Erfolgsaussichten nach Projektende

das Projekt war nicht darauf ausgelegt gewesen, wirtschaftlich-profitable Ergebnisse und Nachhaltigkeit zu generieren.

-wissenschaftliche und/oder technische Erfolgsaussichten nach Projektende und wissenschaftliche und wirtschaftliche Anschlussfähigkeit für eine mögliche notwendige nächste Phase bzw. innovatorischen Schritte zur erfolgreichen Umsetzung der Ergebnisse

Ein großer Teil der initial gestellten Fragestellungen konnte beantwortet werden. Es ist geplant, die Ergebnis konkret in Kooperationen mit Impffirmen einfließen zu lassen und somit das primäre Ziel, die Entwicklung eines Impfstoffes gegen Hepatitis C, weiter zu verfolgen.

4. Arbeiten, die zu keiner Lösung geführt haben

keine

5. Präsentationsmöglichkeiten für mögliche Nutzer

Das Projekt war als Grundlagenprojekt ausgelegt. Der mögliche Nutzen ergibt sich in Zukunft gegebenenfalls darin, dass Therapieplanungen von Patienten mit chronischer und akuter Hepatitis C und individuelle Berechungen des Therapieansprechens die Ergebnisse dieses Projektes mit berücksichtigen werden. Da antivirale Therapie auf dem Boden pegylierten Interferonen und Ribavirin nach wie vor sehr teuer und nebenwirkungsreich sind, gilt es, die vorhanden Möglichkeiten zur Vorhersage eines Therapieerfolges oder -Misserfolges weiter stetig zu verbessern, um unnötige Therapien zu vermeiden.

6. Einhaltung der Ausgaben- und Zeitplanung

Es wurden alle beantragten Mittel in dem beantragten Zeitraum abgerufen und komplett verbraucht. Der detaillierte Mittelabrufplan ist in der der Hep-Net Zentrale vorhanden.

IV. Kurzfassung

Therapy of acute and chronic hepatitis C virus infection is based on type I interferons in combination with ribavirin. Besides direct antiviral effects of IFN alpha, modulation of immune responses are thought to be important for successful and sustained clearance of HCV. The complex network of antiviral immunity against HCV consists of humoral immune responses, T cell responses, adaptive immunity mediated by NK cells and different subsets of dendritic cells. The investigations were coordinated with project 10.2.1 (Gruener, Pape) to avoid overlaps and to use synergistic effects between the projects.
Results of our project can be summarized as follows:
1) Dendritic cells: In contrast to HIV and measles virus studies on monocyte-derived DCs, freshly isolated myeloid DCs of patients with hepatitis C do not show an increased but a completely abolished cytotoxic activity. The impaired DC cytotoxicity could represent a novel mechanism for the increased prevalence of autoimmunity in HCV infection.
2) Natural Killer cells: a.) NK cell subsets show different TRAIL-expression patterns in response to type I interferons. B) NK-cells show several phenotypic changes in acute hepatitis C virus infection which are linked to activation and cytotoxic activity. Our findings differ to recent data on chronic hepatitis C. Most killing inhibitory receptors were not altered but NKG2A expression may be increased by interferon alpha therapy. However, no clear NK-cell pattern could be linked to virological response to IFN treatment.
3) HCV-specific T cells: (i) We demonstrated the development of HCV-specific T cells in HCV-exposed individuals after needle stick injury indicating subinfectious exposure to HCV. T-cell immunity against HCV may contribute to the low prevalence of HCV in medical healthcare professionals in Western countries. (ii) Differentiated CD27- effector CD4+ CTLs can be detected in patients with viral hepatitis. In particular in patients with more advanced liver disease, the accumulation of perforin-positive T cells with age could be one correlate for the more severe course of viral hepatitis in elderly individuals. (iii) Kinetics of HCV-specific T-cell responses are heterogeneous in interferon-treated patients with acute hepatitis C. The decline of T-cells might be a consequence of both apoptosis and homing. The balance between cell death and regulation of chemokine receptors might lead to different long-term outcomes. (iv) HCV-specific memory cells can be induced by peptide vaccination and that a reversion of functional impaired phenotypes by therapeutic vaccination is possible in chronic HCV infection. (v) Clearance of low levels of HCV viremia is possible in the absence of a strong adaptive immune response which might explain the low seroconversion rate after occupational exposure to HCV.