10th Banff Conference on Allograft Pathology
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An Update

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The Banff Conferences on Allograft Pathology

Date	Venue	Comments
1991	Banff	Kidney only
1993	Banff	Kidney only
1995	Banff	First liver session. Acute rejection - diagnosis & grading. Banff consensus paper – Hepatology 1997.
1997	Banff	Chronic rejection – diagnosis & staging.
1999	Banff	Chronic rejection – diagnosis & staging. Banff consensus paper – Hepatology 2001.
2001	Banff	Late biopsies – role in identifying graft dysfunction
2003	Aberdeen	Late biopsies - role in identifying graft dysfunction
2005	Edmonton	Late biopsies - role in identifying graft dysfunction Banff consensus paper – Hepatology 2006.
2007	La Coruna	Late biopsies – role in identifying tolerance
2009	Banff	Late biopsies – role in identifying tolerance

10th Banff Conference on Allograft Pathology
Liver Sessions

- 9 speakers, 2 afternoons
- 30 (=15) minutes per talk

(1) Introduction, Challenges and Focus of Meeting

Jake Demetris, Pittsburgh

Achieving tolerance in long-term survivors

- "True" tolerance – no detectable immune response
  - Induced using specific therapeutic approaches (early post-transplant)
- "Operational" tolerance – minimal immune response (not clinically significant)
  - Withdrawal of immunosuppression from patients with stable graft function (usually > 2 years post-transplant)

Questions to be addressed:

1. Should tolerant patients be routinely subjected to liver biopsy?
2. What long-term histological changes are permissible?
3. Does the liver allograft participate in mechanisms of tolerance?
4. How should biopsies be studied
   - Conventional histology
   - Multiplex immune imaging - simultaneous detection of multiple lymphoid subsets (see also AASLD Abstract 1234)

Weaning of Immunsuppression in LT-Patients with Stable Graft Function

(Mazariegos 1997 2003, Devlin 1998, Takatsuki 2001, Pons 2003, Eason 2005, Tisone 2006)

Role of Liver Biopsy (from SGH talk Banff 2007)

- Pre-weaning (baseline)
  - Mainly taken to exclude rejection
  - Detailed histological findings lacking
  - Time of biopsy in relation to stopping immunosuppression unclear

- Post-weaning
  - Mainly taken to investigate graft dysfunction + confirm rejection
  - Most cases of rejection are mild and respond to immunosuppression
  - Some cases have mild bile duct atypia/atrophy/ duct loss (Mazariegos 1997, Tisone 2006,Koshiba 2007)
    - None considered sufficient to warrant a diagnosis of chronic rejection

(2) Immunological aspects of HCV, autoimmunity and liver allograft acceptance

Geoff McCaughan, Sydney, Australia

- Liver has special properties that facilitate interactions between lymphocytes and hepatocytes
  - Low flow system in sinusoids
  - Lymphocyte occupies full diameter of sinusoid
  - Lymphocyte projections extend through fenestrations in sinusoids & come into direct contact with hepatocytes
  - Unique system in which immunologically naïve cells come into direct contact with hepatocytes

Animal models to address 3 questions:

1. Where does lymphocyte activation occur to cause rejection (or lack of activation to produce tolerance)?
   - T cells activated by Ag presentation in lymph node subsequently entering liver cause hepatitis
   - If lymph node entry blocked T cells still enter liver but don't cause hepatitis
2. Is antigen presentation by hepatocytes sufficient alone for immune activation to cause rejection/hepatitis?
   - NO
3. What happens to T cells activated in liver that don't cause liver damage?
   - Impaired CTL function and premature death
   - T cells actively invade into hepatocytes (3-D studies), move around and degraded in endosomes

(3) The spectrum of findings in protocol and indicated biopsies in long-term liver allograft recipients with an emphasis on diagnostic challenges

Stefan Hubscher, Birmingham, UK

Main Pathological Changes in Biopsies >12 Months Post-transplant

- **Rejection**
  - Less common than in early post-transplant period
  - May have different histological features
  - Worse outcome
- **Recurrent disease**
  - General issues
  - Assessment of biopsies from HCV-positive individuals
- **De novo disease**
  - General issues
  - De novo autoimmune hepatitis
- **Other findings in late biopsies**
  - “Idiopathic” chronic hepatitis
  - Vascular/structural abnormalities

"Idiopathic" Chronic Hepatitis

- Common(est) diagnosis in late post-transplant biopsies
  - 20-30% of biopsies (>12 months) in Bham
  - Prevalence increases with time - up to 60% by 10years (Evans 2006)
- Problems with terminology
  - interface hepatitis, "de novo" AIH, graft inflammation (non-specific), late rejection (with hepatitic features),
- Emerging evidence to suggest that (allo)immune mechanisms important, particularly in children
  - 70-80 % of patients have auto-antibodies (Evans 2006), but don't fulfil criteria for "de novo" AIH (LFTs normal/near-normal)
  - Other cases have features suggestive of chronic rejection - 55% in Montreal (Herzog 2008)
- Associated with development of progressive fibrosis
  - By 10 years 50-70% have bridging fibrosis or cirrhosis
- Fibrosis progression may be prevented by immunosuppression
  - 2 studies (Birmingham – Haller AASLD 2009, Kyoto - Miyagawa-Hiyashino Transpl Int 2009)

(4) Complete withdrawal of immunosuppression in liver transplantation

George Mazariegos, Pittsburgh, USA

Review of previously published series

Pittsburgh (Mazariegos Transpl Immunol 2007)

- 18/95 (19%) patients complete withdrawal (>2yrs off immunosuppression)
- Highly selected:
  - > 5 years post-LT
  - No rejection episode in preceding 2 years
  - Absence of acute or chronic rejection in baseline biopsy

Kyoto (Koshiba Transpl Immunol 2007)

- 85/675 (12.6%) of **all** paediatric patients successfully weaned
  (more details of Kyoto experience presented later)

Rejection = main cause of weaning failure in both studies

- Usually responds well to immunosuppression with no adverse long-term effects

Complete withdrawal of immunosuppression in liver transplantation

Histological Findings in Post-Weaning Biopsies

Acute rejection (usually respond to immunosuppression)

Other findings (includes protocol biopsies)

- Portal inflammation +/- interface hepatitis
- Bile duct atrophy/focal duct loss (not sufficient to diagnose chronic rejection)
- Nodular changes
- Recurrent disease (autoimmune disease may be “unmasked”)
- Fibrosis (may be more severe in tolerant than non-tolerant – Koshitomi 2009)

CONCLUSION

- Natural history and pathology of tolerant grafts without immunosuppression is uncertain
- Protocol biopsies planned at 1,3, 5 years

(5) Biomarker prediction of successful weaning of liver allograft recipients

Alberto Sanchez-Fueyo, Barcelona, Spain

Review of previous studies (Martinez-Llordella 2007,2008)

- 28 patients with “operational tolerance” (5 centres)
- Studies carried out at baseline and 12 months after weaning complete

Factors predicting tolerance

- Tolerant patients have more potential Tregs (CD4+/CD25+/FoxP3+ and Vdelta1+) in peripheral blood (BUT – too much overlap to be clinically useful)

- Gene microarray profiling more discriminatory:
  - 34 genes differentially expressed in tolerant cases
  - Genes code for gamma-delta T cells, NK cells and cell proliferation arrest proteins

Biomarker prediction of successful weaning of liver allograft recipients

Alberto Sanchez-Fueyo, Barcelona, Spain

Prospective Study

- 18/102 patients enrolled became tolerant

  (no rejection, 12 months follow-up)

Factors predicting successful weaning:

1. Time post-LT (3-6yrs = < 10%, > 10 years = > 50%)
2. Low CNI levels at baseline
3. Gene profiling studies (increased T regs. NK cells, delta 1 T cells)

- Gene profiling studies also carried out on liver tissue – poor discrimination (? diluting effect of non-lymphoid tissue)

(6) “Plasma Cell Hepatitis” in Protocol Biopsies

Mylene Sebagh, Paris, France

- Histological features suggestive of AIH in patients transplanted for other diseases (“de novo AIH”) – present in 35/1097 grafts (1999-2005)

- Differential diagnosis
  - Late rejection
  - “idiopathic” post-transplant hepatitis
  - De novo HBV/HCV

- Issues with terminology
  - Descriptive term (“plasma cell hepatitis”) may be more appropriate than one which implies known aetiology (“de novo AIH”)

    (“plasma cell hepatitis”/ “de novo AIH”, idiopathic CH and late rejection may be part of a spectrum of immune-mediated graft damage)

- Additional (non-histological) diagnostic criteria required
  - Problems with applying IAHG scoring system to liver allograft recipients

    (e.g. recurrent AIH without auto-antibodies, auto-antibodies common in children without evidence of graft dysfunction)

"Plasma Cell Hepatitis" in Protocol Biopsies (n =35)

Mylene Sebagh, Paris, France

Histological Findings:

- Interface hepatitis - 100% (A1 – 51%, A2/3 – 49%)
- Centrilobular inflammation - 87% (A1 – 24%, A2/3 = 76%)
- Lobular inflammation - 60% (A1 – 80%, A2/3 = 20%)

Clinical/Biochemical Findings

- Abnormal LFTs 31/35
- Auto-antibodies 16/29
- Raised IgG 26/31

Response to treatment

- Increased immunosuppression (n=21)
  - 14 LFTs improved, 7 LFTs still abnormal but biopsy better
- Reduction in immunosuppression (n=14)
  - - many also had improved LFTs

Changes proposed to IAHG Scoring System

- (see also ILTS Meeting, July 2008, Abstract 61)

(7) Immunosuppressive management decisions in long-term liver allograft recipients
Relationship to biopsy findings

James Neuberger, Birmingham U.K.

Survival post-LT

- For patients alive at 1 year, risk of graft loss has not changed during the past 10-15 years

  (improved survival due to better results during first year)

Average life expectancy

- For adults less than equivalent UK population (22 vs 29 years)
- Worse outlook for children

Most late deaths are related to complications of immunosuppression

- CVS disease, renal failure, diabetes, malignancy

Consider benefits /risks of immunosuppression to maintain healthy graft

Role of Protocol Liver Allograft Biopsies

Survey of 35 liver transplant centres (Mells & Neuberger 2008)

- 23 (65%) protocol annual biopsies for HCV-positive patients
- 9 (25%) protocol biopsies for other patients
- Reasons for discontinuing protocol biopsies
  - Risk of potentially serious complications, cost, problems with histological interpretation, non-invasive alternatives (e.g. to assess fibrosis), findings don't influence management

Late Protocol Biopsies in the Liver Allograft (Mells, Hubscher & Neuberger 2009)

- 235 biopsies > 1yr post –LT. All had normal LFTs at time of biopsy
- 76% abnormal histologically
  - "idiopathic CH"(33%) , recurrent disease (23%), fatty liver (14%), other (5%)

76/235 cases had change in immunosuppression after biopsy

- 11 increased (active inflammation in protocol biopsy)
- 58 reduced (lack of inflammation in protocol biopsy)
- 7 switched to CNI-sparing regime (active inflammation and renal impairment)

(8) Weaning immunosuppression from long-surviving live donor liver allograft recipients with prospective biopsy monitoring: Preliminary results of the Immune Tolerance Network Pediatric experience

Sandy Feng, San Fransisco, USA

20 patients from 3 centres (UCSF, Chicaho, Columbia)

Inclusion Criteria

- Living donor, > 4 years
- Good renal function
- No auto-antibodies
- Histology – no evidence of AR or CR, Ishak fibrosis score ≤ 2

Outcome

- 12 children had successful withdrawal (good graft function > 12 months post-weaning)

Preliminary results of the Immune Tolerance Network Pediatric experience Histological Findings

Baseline biopsies

- NRH and space of Disse fibrosis common
  - no obvious clinical significance or predictive value
- Mild portal inflammation (vs none) – predictive for weaning failure
- Ishak fibrosis stage 3 (n =1, excluded from weaning)

Failed Cases (during/after immunosuppression withdrawal, n = 7)

- Increased numbers of portal inflammatory cells
  - AR mod (1), AR mild (1), AR indeterminate (5)

Following Successful Weaning

No significant changes in biopsies obtained at 4-6 weeks (n =7) and 2 years (n=4)

(9) Clinical, immunological, and pathological observations in tolerant liver allograft recipients

Takaaki Koshiba, Kyoto, Japan

Selection Criteria:

- Normal graft function > 2 years post-LDLT
- > 1 year rejection-free
- (no protocol biopsies ? before 2003)

Outcome

- 85/675 (12.6%) operational tolerance
- 85/191 patients in whom weaning was attempted

Factors correlating with tolerance (compared with rejection during/after weaning)

- No rejection episodes during 1st month
- HLA B mismatch
- Female donor
- Higher numbers of FoxP3 postive T cells (Tregs) in peripheral blood and liver

Tolerant Patients vs Patients Maintained on Immunosuppression
Further Studies Comparing Histological Findings
(see also Yoshitomi Transplantation 2009: 87: 606-614)

Protocol Biopsies (since 2003) at 1, 2, 5, 10 years

	Gr-Tol (n=29)	Gr-IS (n=29)
Age (mean)	1.0	4.2
Time of biopsy (months post-LT)	121	52
Mean fibrosis score (Ishak)	1.6	0.9
FoxP3 + cells in liver biopsy/mm^2	70	23

More fibrosis in tolerant grafts (also perivenular) – Possible mechanisms

1. Antigen independent – e.g. time post-transplant
2. Antigen dependent - T regs may switch cytokine profile from a pro-inflammatory (TH1) to a pro-fibrogenic (TH2) phenotype

BUT

- NO difference in CD4/CD8 cells in 2 groups
- NO correlation between Foxp3 cell number and fibrosis

Kim Solez

http://www.kimsolez.com/pro_profile.html

Banff 2009 – The Year of the Hat