HAEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT)

"Conditioning Regimen"

(myeloablative chemotherapy)

- Destroys the host bone marrow making space for the transplanted graft
- Destroys the tumor (autoreactive) cells

↓

Infusion of the "immune cells enriched" donor graft into the host

TRANSPLANTED GRAFT

- From an aspirate of bone marrow
- From peripheral blood into which SC have been mobilized
- From placental cord blood obtained from the umbelical vein
- Graft SCs repopulate the host haematopoietic system
- It contains mature immune cells including T cells, NK and DC

HLA MISMATCH OBSTACLES HCT

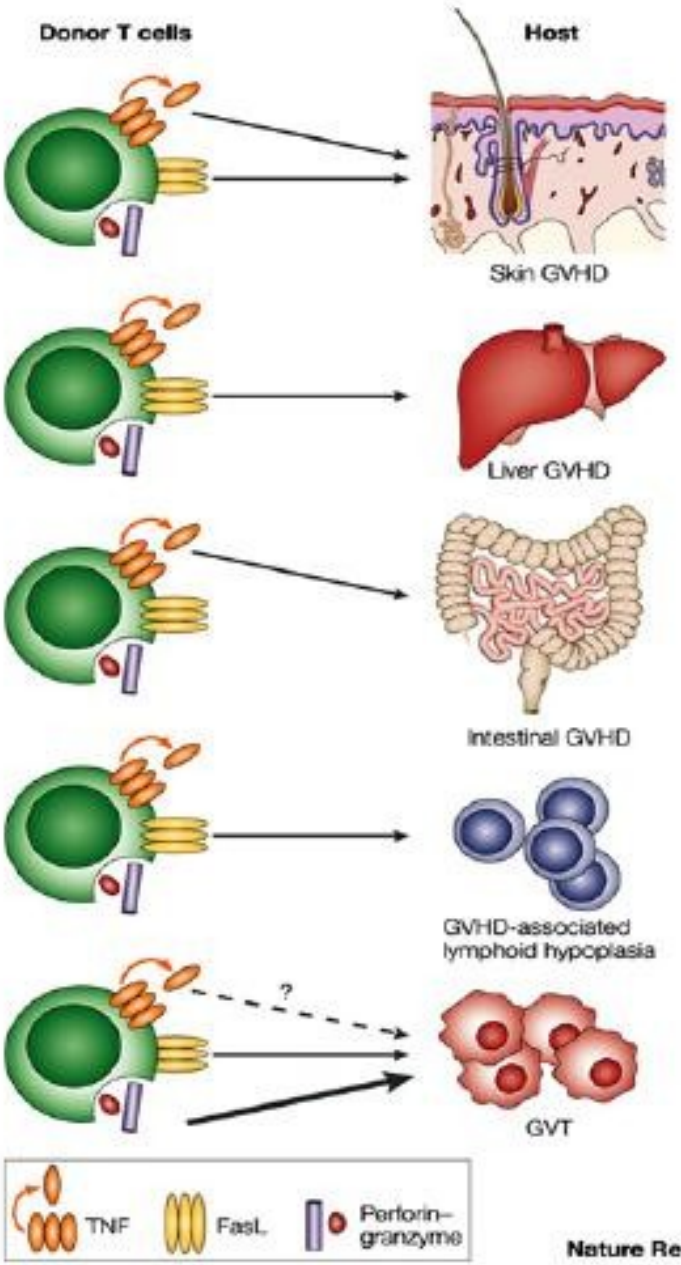
- HLA identical donor
  - Sibiling donor
  - Unrelated donor
- Partially mismatched donors
  - Single class I or class II mismatch
  - Multiple class I or class II mismatches
- HLA haploidentical donor (sharing at least one allele at each of the polymorphic HLA class I and II genes)

PROS AND CONS OF ALLOREACTIVITY

- GVHD
- It facilitates the engraftment cleaning the host bone marrow and preventing rejection
- Graft Versus Tumor (GVT) activity

GRAFT VERSUS HOST DISEASE

- Conditioning regimen are toxic to many tissues
  - Skin
  - Gastrointestinal tract
  - Liver
- Tissue injury is mediated by:
  - Increased intestinal permeability
  - Release of Th1 cytokines by host cells creating an inflammatory cytokyne milieau
    - Activation of donor and host APCs
    - T cell activation and IL-2 sustained clonal expansion



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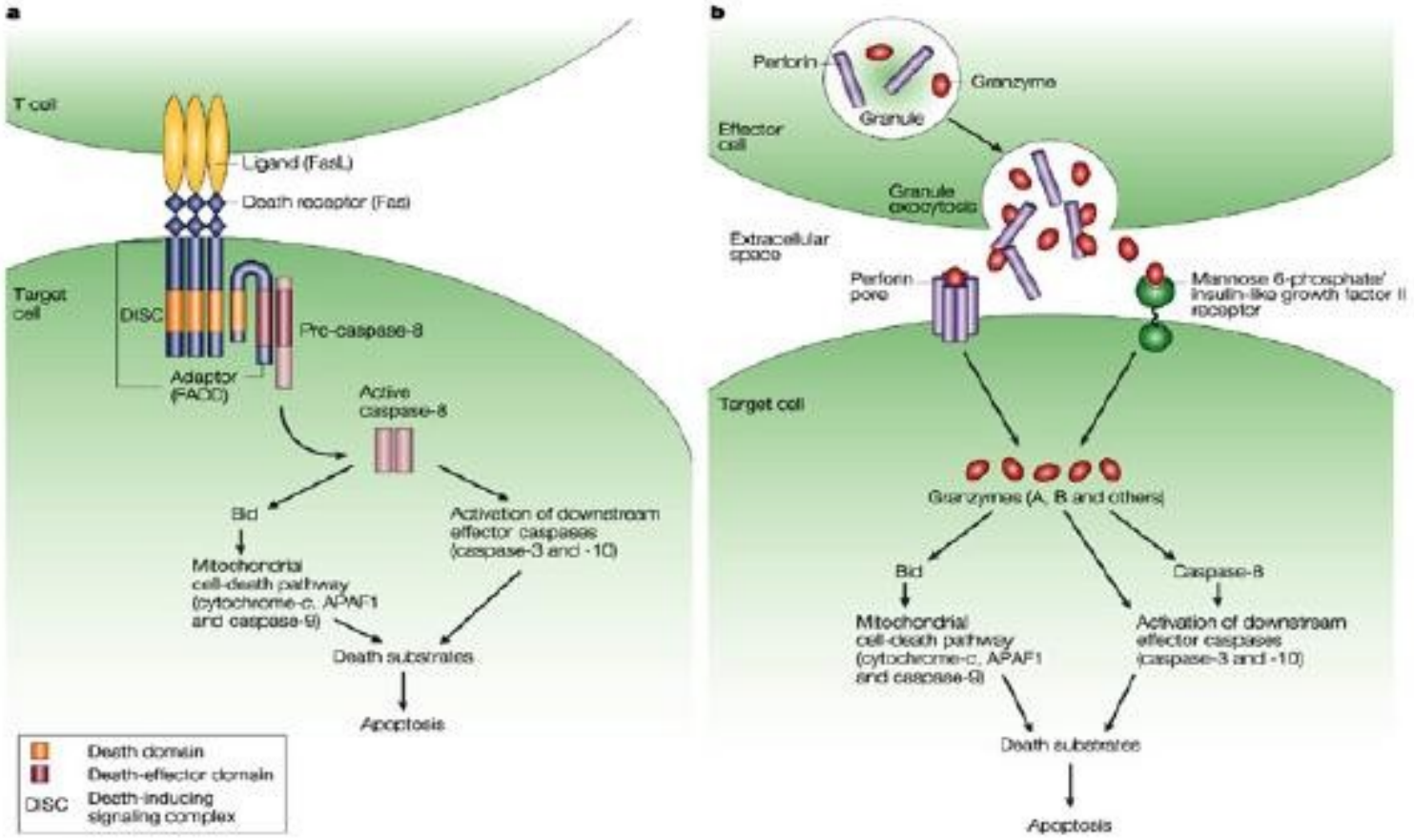
GRAFT VERSUS HOST DISEASE

- Tissue injury is mediated by:
  - Increased intestinal permeability
  - Release of Th1 cytokines by host cells creating an inflammatory cytokyne milieau
    - Upregulation of MHC molecules on donor and host APCs
    - T cell activation and IL-2 sustained clonal expansion
    - Activation of mononuclear phagocytes and NK cells
  - Activation of cytolytic pathways
    - FAS/FASL
    - Perforin/Granzyme

CYTOLOYTIC PATHWAYS



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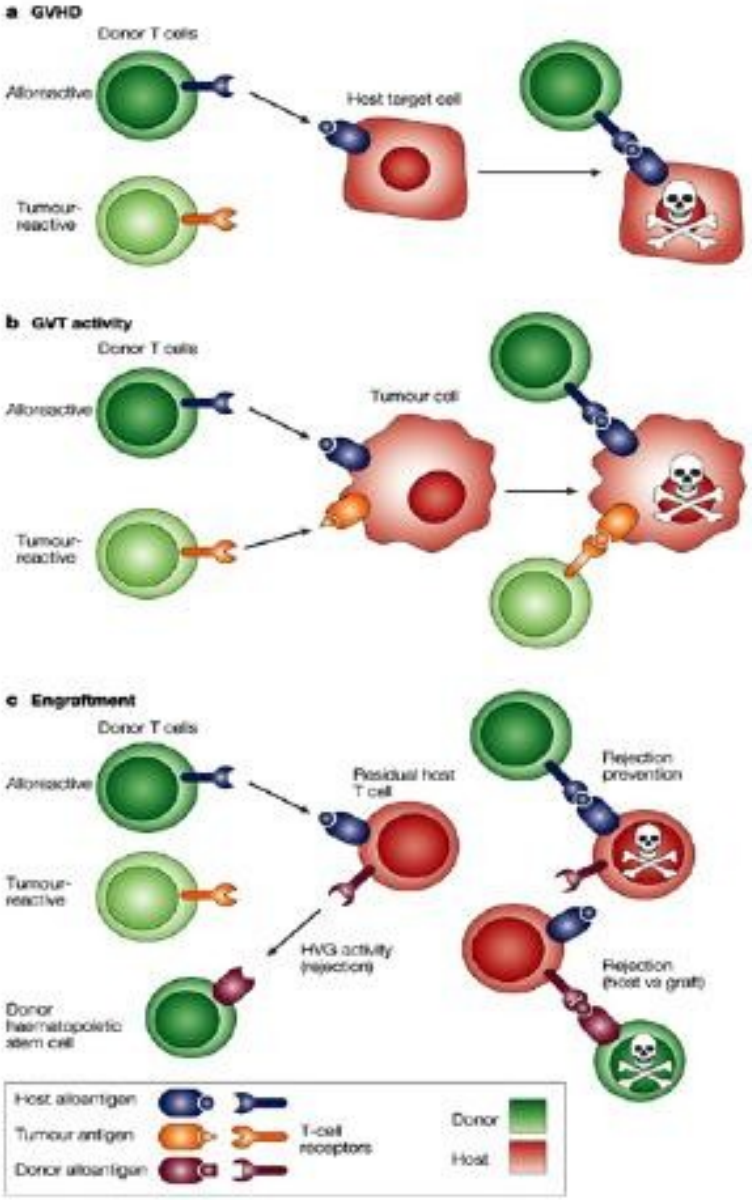
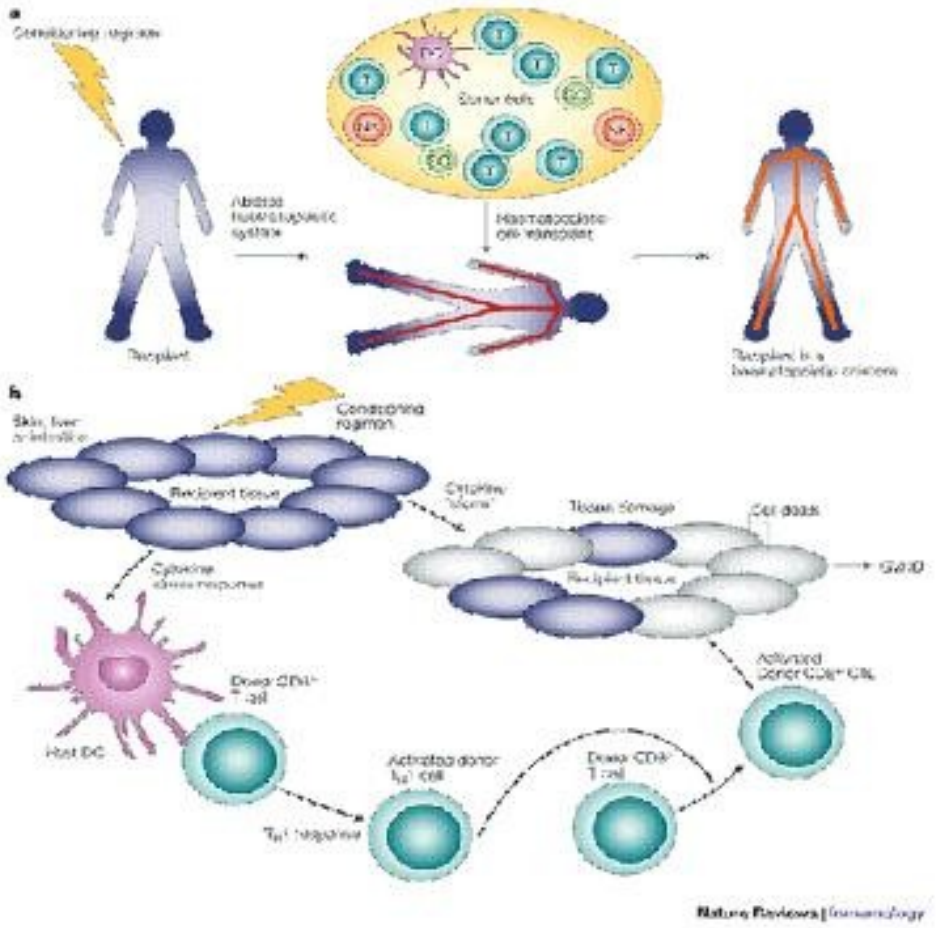
MINOR HISTOCOMPATIBILITY ANTIGENS

- GVHD is initiated by donor T cells that recognize a subset of host peptides, called minor histocompatibility antigens (miHAs), derived from from differences in the peptides that are presented by the same HLA class I allotype between recipient and donor
  - Polymorphisms of other, non HLA, proteins
  - Differences in the level of expression of proteins
  - Genome differences between males and females (e.g. H-Y antigens)


a GVHD
Donor T cells
Alloreactive
Tumour-reactive
Host target cell
b GVT activity
Donor T cells
Alloreactive
Tumour-reactive
Tumour cell
c Engraftment
Donor T cells
Alloreactive
Tumour-reactive
Donor haematopoietic stem cell
Residual host T cell
HVG activity (rejection)
Rejection prevention
Rejection (host vs graft)
Host alloantigen
Tumour antigen
Donor alloantigen
T-cell receptors
Donor
Host

Haematopoietic-cell transplantation and graft-versus-host disease



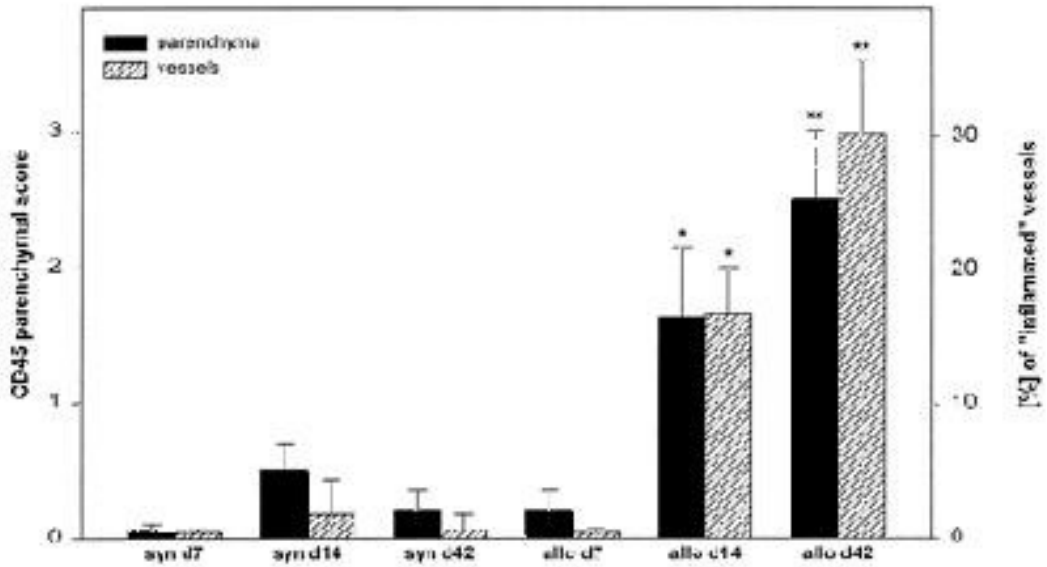
CNS as immunologically priviliged site

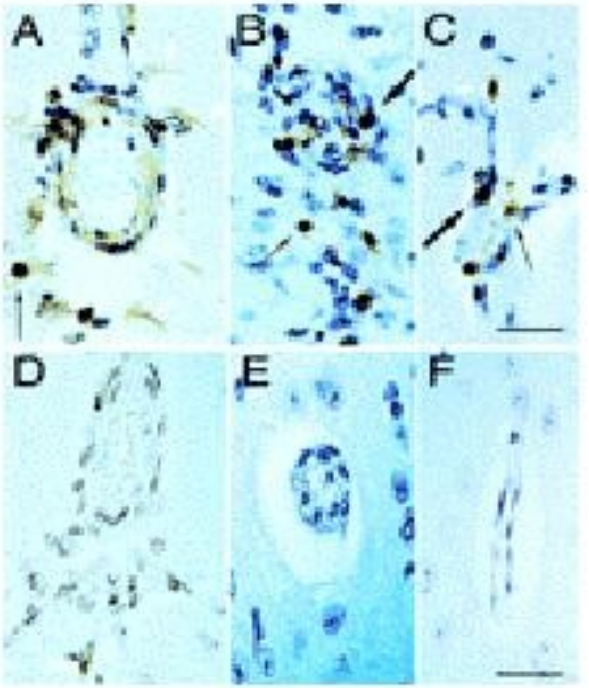
- CNS is rarely involved by GVHD
- Anatomical separation between blood and CNS (BBB)
- CNS structures are hidden from the immune system and thus do not suffer for direct effects of conditioning regimen
- Lack of costitutive MHC expression and professional APC
- Immunosuppressive microenvironment maintained by cytokines released by CNS cells
- Lack of lymphatic drainage
- Lack of immunological surveillance by T-cells

Neurological complications following BMT

- Angiitis (Padovan et al, 1999; Ma et al, 2002)
- Leukoencephalopaty and ADEM (Solaro et al, 2000; Tomonari et al, l 2003)
- Immune mediated myelopathy (Openshaw et al,1995)
- GBS and CIDP (Wen et al, 1997; Solaro et al, 2000; Nagashima et al, 2002)
- Neuromyotonia (Liguori et al, 2000)
- Polymyositis (Tse et al, 1999)
- Vasculitic Neuropathy (Openshaw et al,1997)
- Miastenia Gravis (Dowell et al, 1999; Tse et al, 1999)
- Stroke
- CNS infections
- Immunosuppressive drugs toxicity
- Metabolic encephalopaties

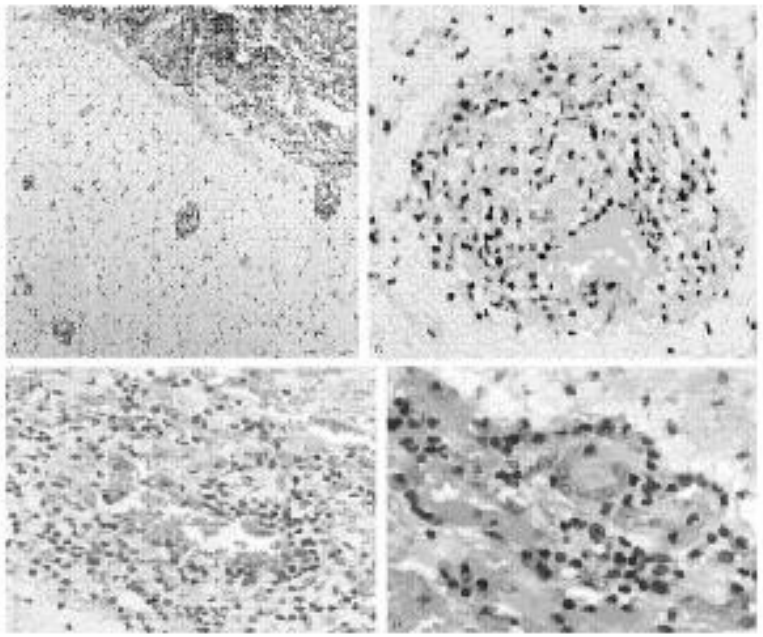
GVHD mediated angiitis

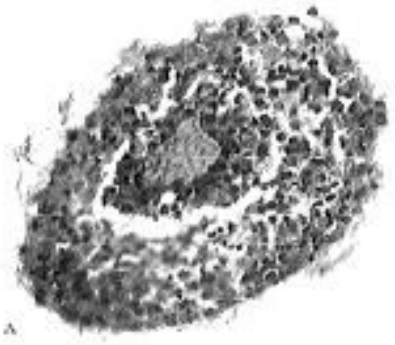


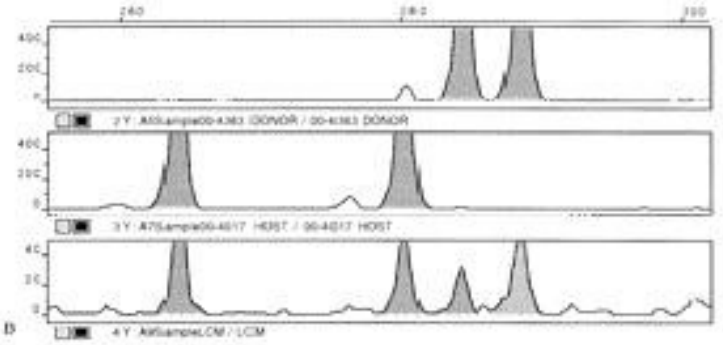


Padovan et al, Neurology 2000

GVHD mediated angiitis







Ma et al, Neurology 2002

Individuals presenting with symptoms suggestive of encephalomyelitis following allogeneic BMT @ DINOG

	Patients
MRI abnormalities	4/6
CSF abnormalities	6/6
Anti-neuronal antibodies	1/6
Detection of lab findings of viral infection	0/6
Systemic GVHD	3/6

Diagnosis of cerebral GvHD

Proposed criteria

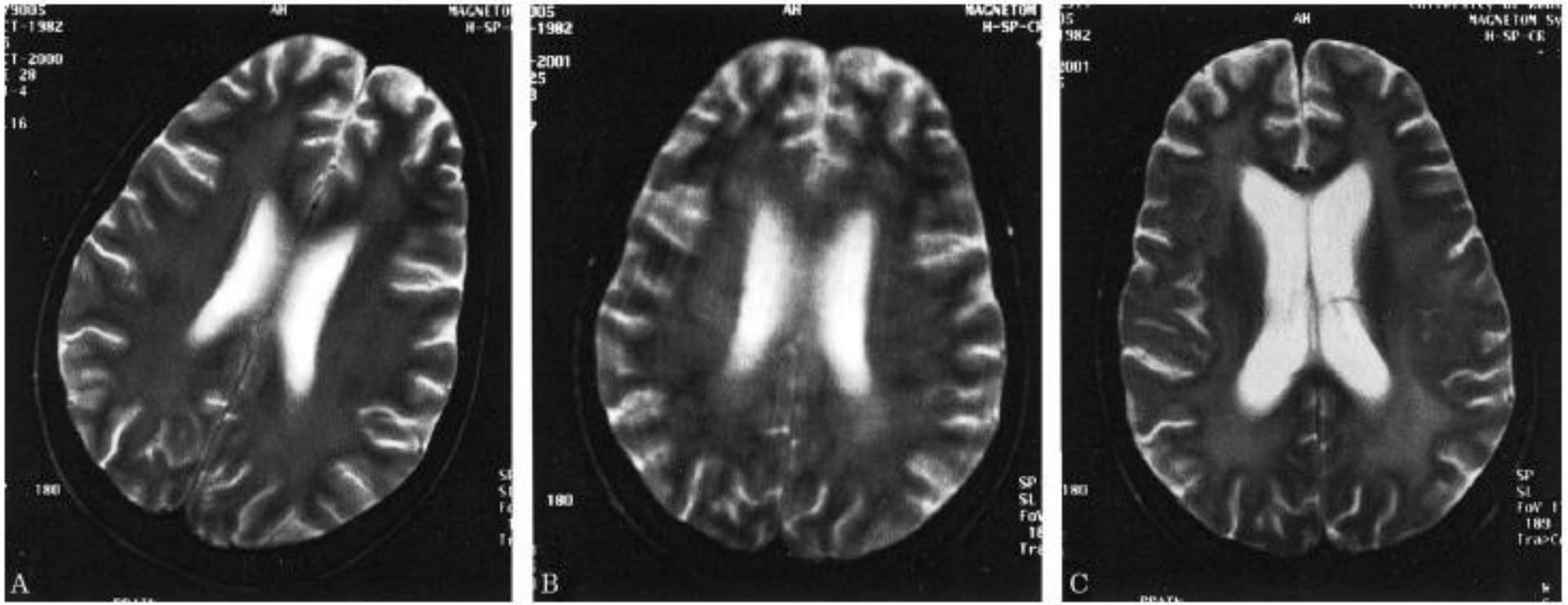
MANDATORY

- Neurological symptoms occurring following BMT
- No direct correlation with administration of immunosuppressive agents
- Exclusion of concomitant CNS infections
- Presence of CSF abnormalities
  - Pleiocytosis
  - Intrathecal synthesis
  - OB in the CSF

ADDITIONAL

- Multifocal MRI abnormalities
- Presence of antibodies anti-neuronal antigens
- Onset following a systemic co-morbid event
- Clinical response to immunosuppressive treatments or worsening following immunosuppressive withdrawal

BRAIN GVHD MRI ABNORMALITIES



GVHD as result of the adoptive transfer of autoimmunity

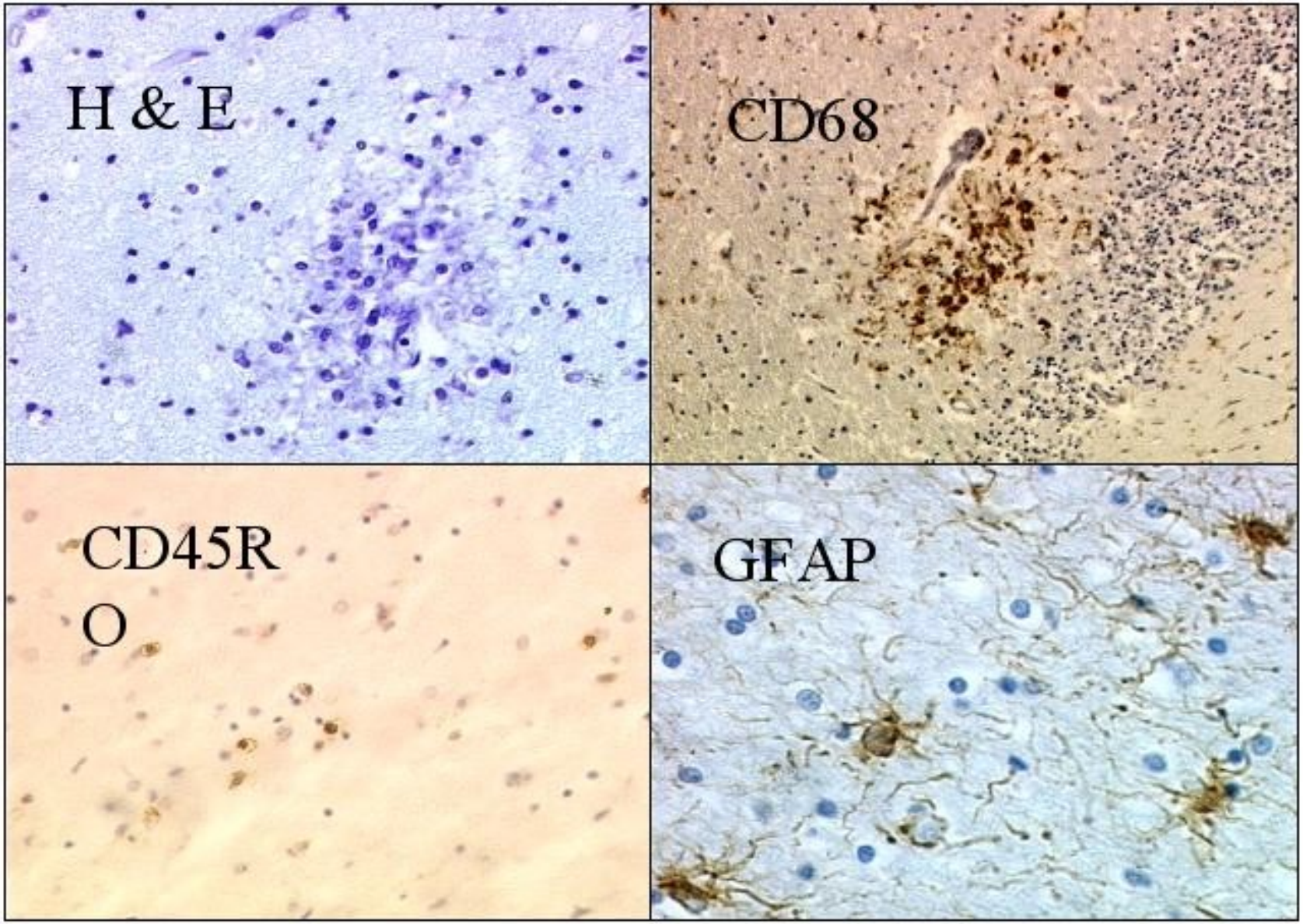
- Vitiligo (Neumeister et al, 2000)
- Lupus (Marmont, 1992)
- T-cell lymphoma (Berg et al, 2001)

CLINICAL HISTORY

- A young male, who underwent BMT for aplastic anaemia, developed intractable myoclonus after the occurrence of head trauma.
- No clinical improvement observed after CyA withdrawal and conventional anti-epileptic as well as anti-myoclonic treatments
- Based on the presence of a single clonal band of IgG in the CSF, high dose iv cyclophosphamide was administered with transient improvement.
- Following interruption of the immunosuppressive therapy, clinical condition progressively worsened to death.

LAB FINDINGS AND IMAGING

- Routine lab tests: normal
- Antibodies anti- CMV, HIV, HTLV 1, HSV 1,2,7 and anti-Yo, -Hu, -Ri, -GAD and anti-gangliosides were not detected both in serum and CSF.
- Reactivity against GluR3 was tested after patient death on the available biological samples.
- Brain MRI (3): normal; EEG (5): normal
- CSF (2): mild pleiocytosis and one single oligoclonal band
- SEP (median nerve): giant cortical potentials
- Flow-cytometry and TCR analysis of PBMC: normal



Histology

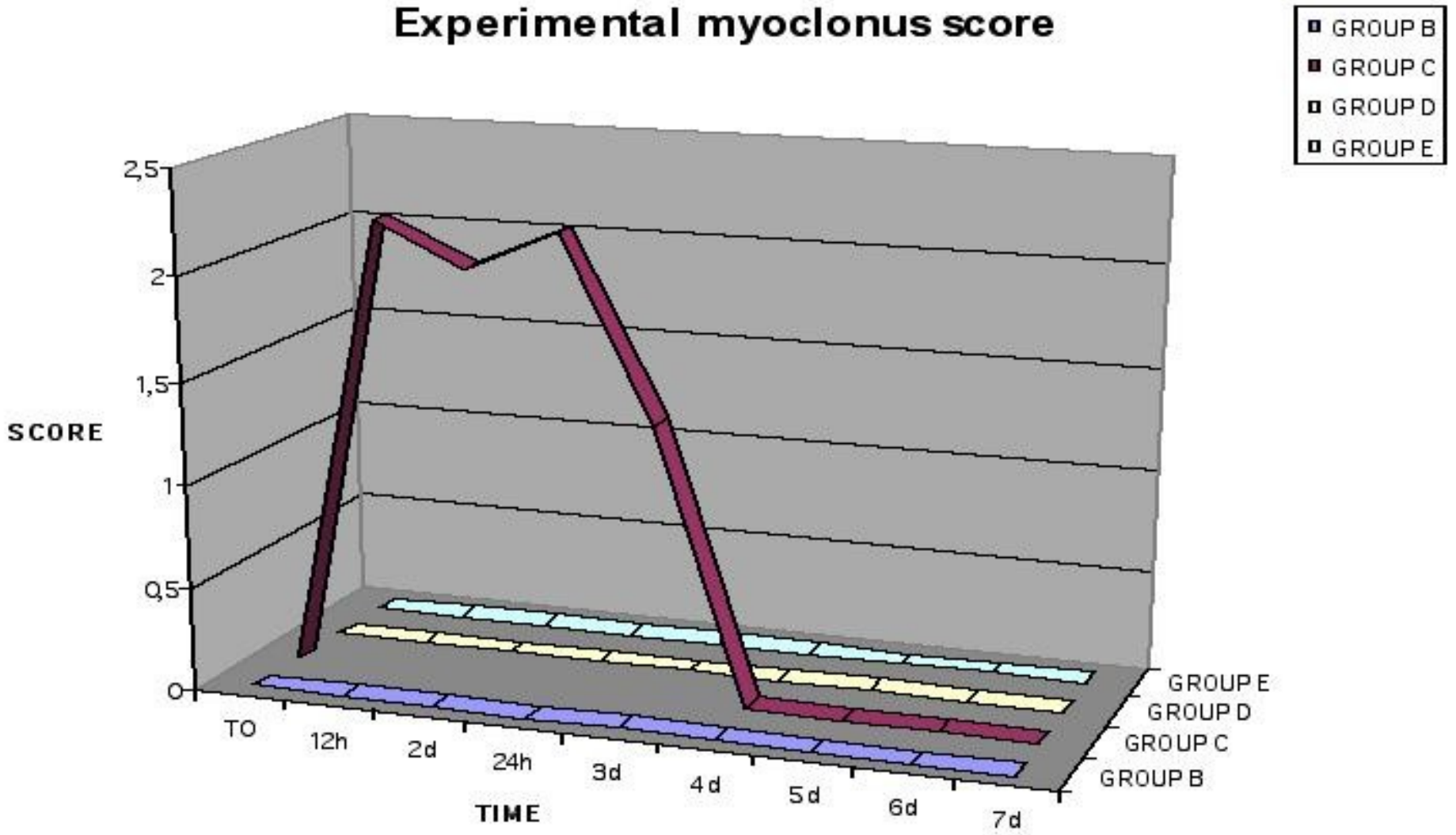
EXPERIMENTAL DESIGN

- IgG were purified by protein A affinity column chromatography
- 21 Sprague Dawley rats were immunized by injection through carotid incannulation
- BBB was opened up by injection with 25% mannitol followed by the administration of CSF or purified IgG
- Evans Blue Dye was injected after mannitol administration in three animals to determine the effect of the technique on BBB integrity
- Myoclonus Rating Scale: 0: no myoclonus; 1: ear twitch; 2: ear and head jerks; 3: ear, head and shoulder jerk; 4: whole body jerk; 5: whole body jerk leading to animal jumping
- E&E, LFB and IHC on brain sections
- Anti-GluR3 antibodies were detected by ELISA
- Reactivities to CNS antigens were examined by Western Blot and Histoblot analysis

EXPERIMENTAL DESIGN

Rats were immunized with:

- Group A) mannitol and EBD alone (3)
- Group B) purified IgG from patient's serum (4)
- Group C) patient's CSF (6)
- Group D) CSF from MS patients (4)
- Group E) CSF from subjects with OND (4)



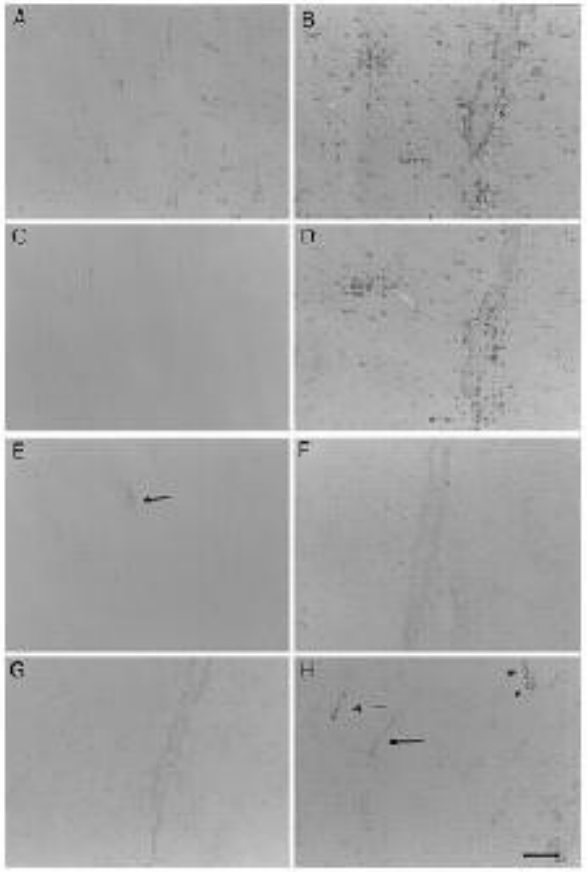
Affected animals showed decreased alertness and altered response to external stimuli. Rhythmical twitching and unintentional movements such as ears, shoulders and head jerks were observed

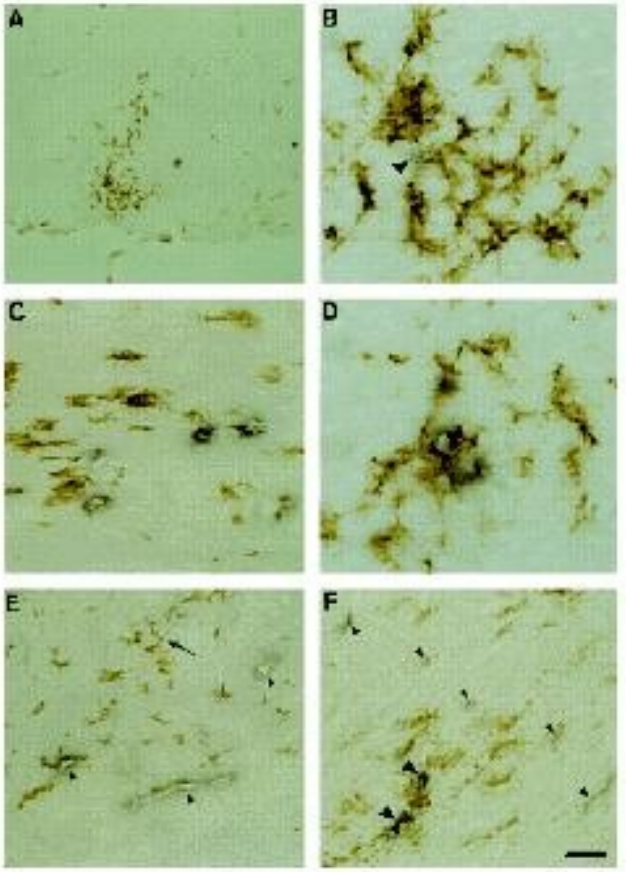
Parenchymal or Meningeal infiltrates in the brain of immunized rats.

	Group A			Group B				Group C						Group D				Group E			
	1	2	3	4†	5	6	7	8	9	10	11	12†	13	14	15†	16	17	18	19	20	21†
P								+++			+++										
M							+	+++	++		+++		++				+			+	

† designates animals died during surgery

ACTIVATED MICROGLIA IN MURINE GvHD





Sedgwick et al,

Anti-GluR3 antibodies titers in serum and IgG

	GluR3A		GluR3B	
	1:10* (1.467)**	1:200* (0.718)**	1:10* (1.380)**	1:200* (0.693)
Recipient serum	2.208	0.857	2.345	1.43
Recipient IgGs	ND	0.960	ND	1.316
Donor serum	1.948	0.552	2.102	0.561
Healthy controls IgGs	ND	0.582	ND	0.624

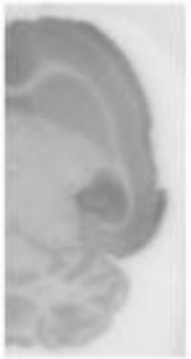
Anti-GluR3 antibodies were measured by ELISA, using two different peptides (GluR3A and GluR3B) at two different sample dilutions (*), and expressed as absorbance at 450 nm.

**Cut-off was defined by the mean OD value obtained from 111 healthy

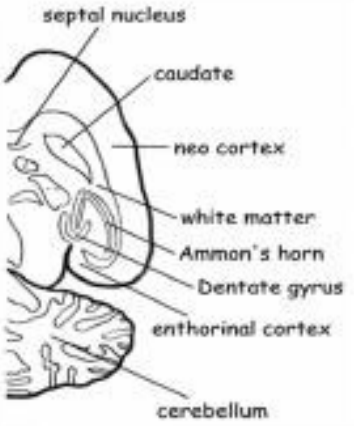
Anti-GluR3 antibodies titers in the CSF

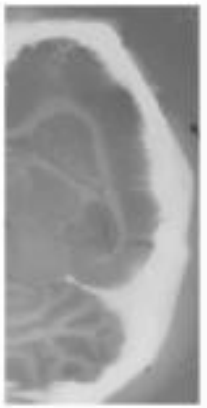
Source	GluR3A
Epilepsy	0.51
Rasmussen E.	0.19
MS	0.310
Patient[a]	1.958

[a]CSF was not tested against GluR3B due to the scarcity of the sample

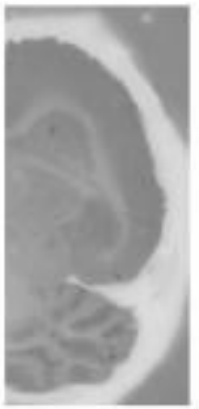


anti-GluR2,3





Donor



Recipient

Control serum

CONCLUSIONS

- Due to the head trauma, the leakage of the BBB led to the access to the brain of immune-competent cells or antigen specific antibodies from the donor capable of recognizing a recipient cerebral antigen, namely GluR3.
- Anti-GluR3 antibodies are most likely the cause of the myoclonic epilepsy, which occurred following allogeneic BMT
- Neurological complications of allogeneic BMT may occur as a consequence of the passive transfer of autoimmunity.

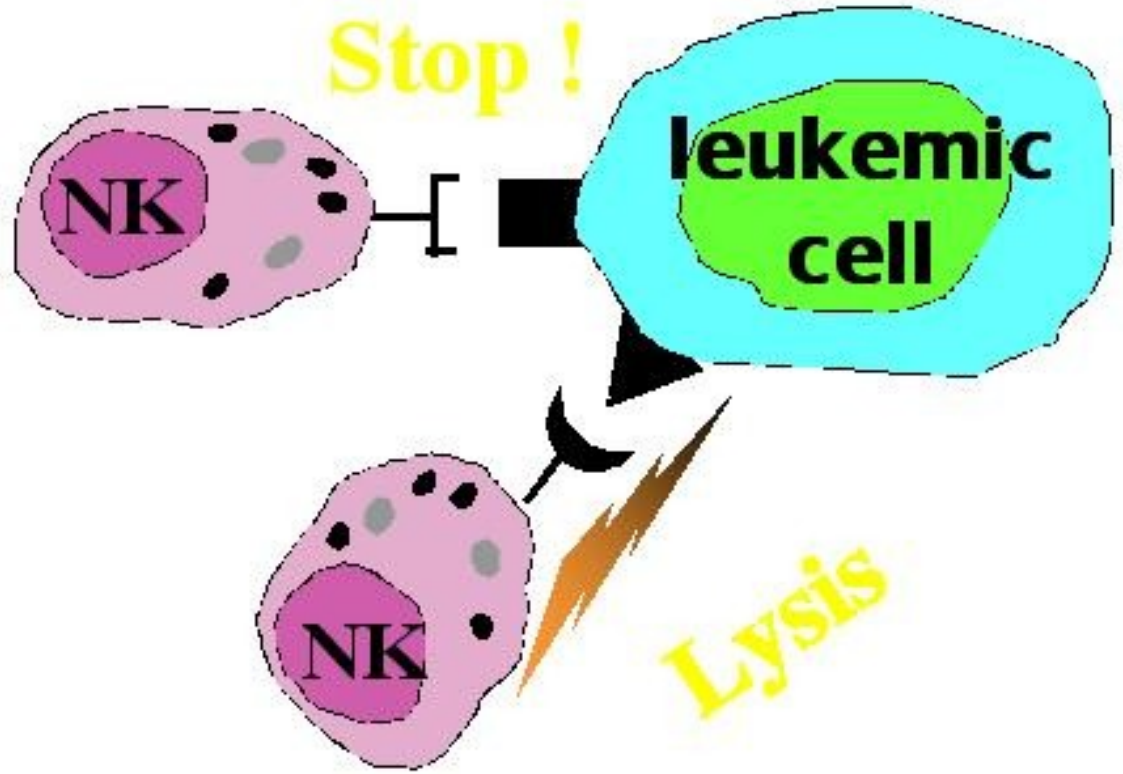
CONCLUSIONS

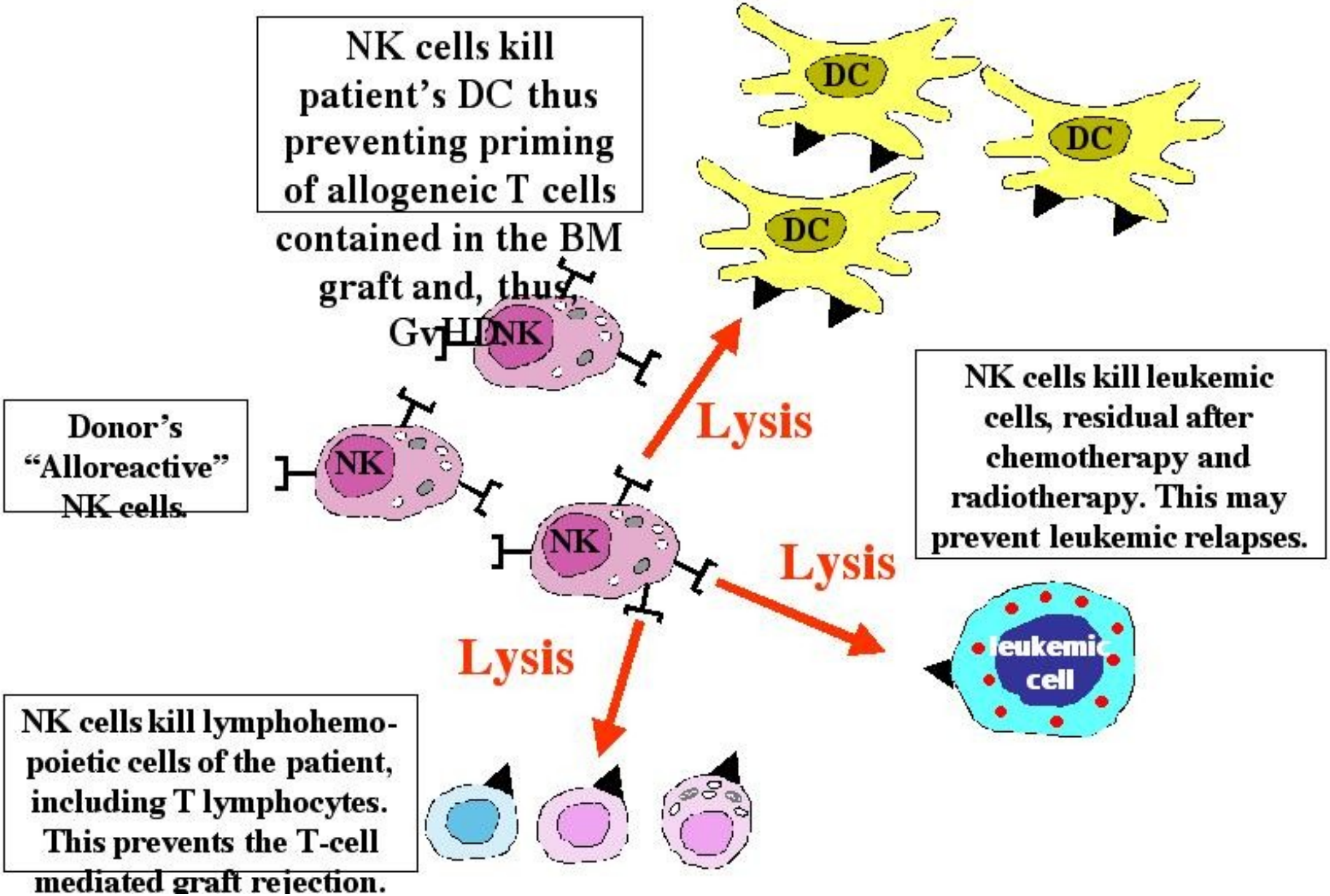
- Due to its immunologically privileged condition, the CNS is rarely subjected to the conditioning regimen's direct toxicity
- CNS involvement may require a co-morbid event (e.g. infection, trauma etc) which may affect the BBB permeability and create the permissive inflammatory milieu necessary for the GvHD to occur
- Due to the absence of specific symptomatic features and the confounding existence of signs associated with long term immunosuppressive treatments (e.g. CyA), immune-mediated neurological syndromes are often under-diagnosed
- Immune-mediated complications of allogeneic BMT may target the CNS more often then previously expected either as part of a systemic GvHD or as a consequence of the passive transfer of autoimmunity.

STRATEGIES FOR HCT: dissection of GVH from GVT effects

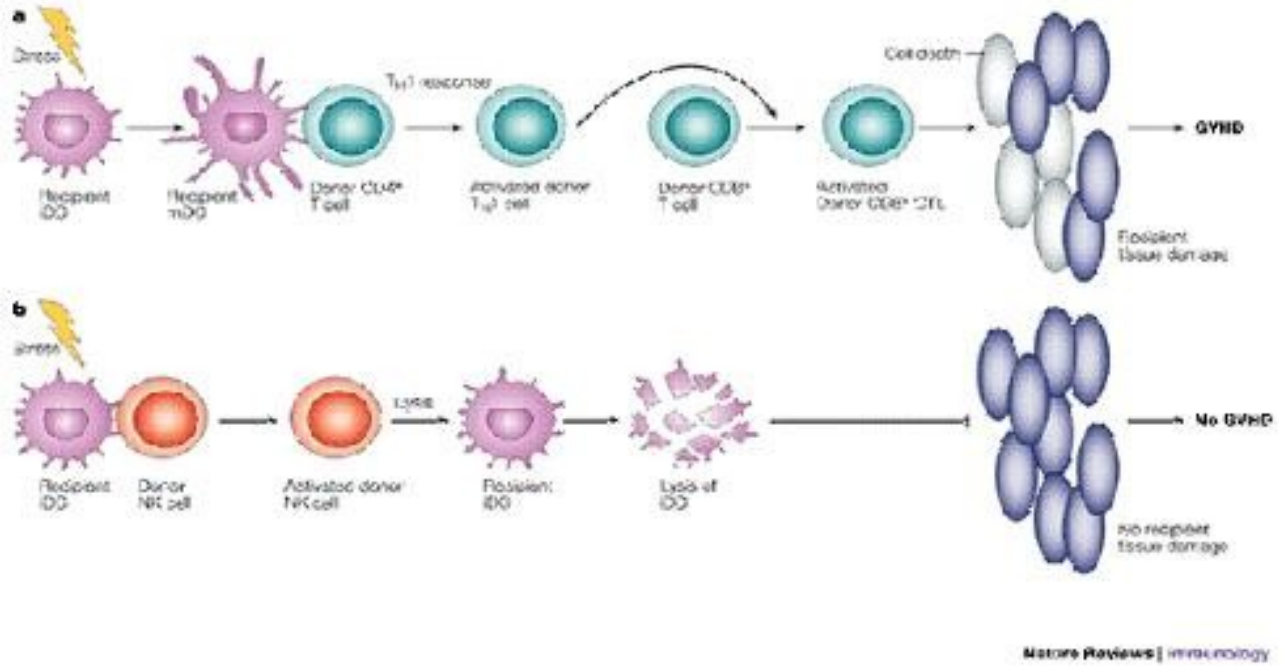
- Intervention on cytokines production
- Long term immunosuppression
- Mini Transplants
- Myeloablation + T cell depletion + DLI
- Mega dose of stem cells
- KIR mismatched alloreactive graft transplant

In an allogeneic setting
(e.g. haploidentical BM transplantation) a fraction of donor's NK cells may express KIR that are not engaged by the HLA-Cl I alleles of the recipient




NK cells kill patient's DC thus preventing priming of allogeneic T cells contained in the BM graft and, thus, GvHD
DC
DC
DC
NK
NK
NK
Donor's "Alloreactive" NK cells
Lysis
Lysis
Lysis
NK cells kill leukemic cells, residual after chemotherapy and radiotherapy. This may prevent leukemic relapses.
leukemic cell
NK cells kill lymphohemo-poietic cells of the patient, including T lymphocytes. This prevents the T-cell mediated graft rejection.

Alloreactive NK cells might prevent GVHD in allogeneic haematopoietic cell transplantation by killing DCs in susceptible tissues



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