B cell phenotyping in Common Variable Immunodeficiency

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Common Variable immunodeficiency Disorders

CVID

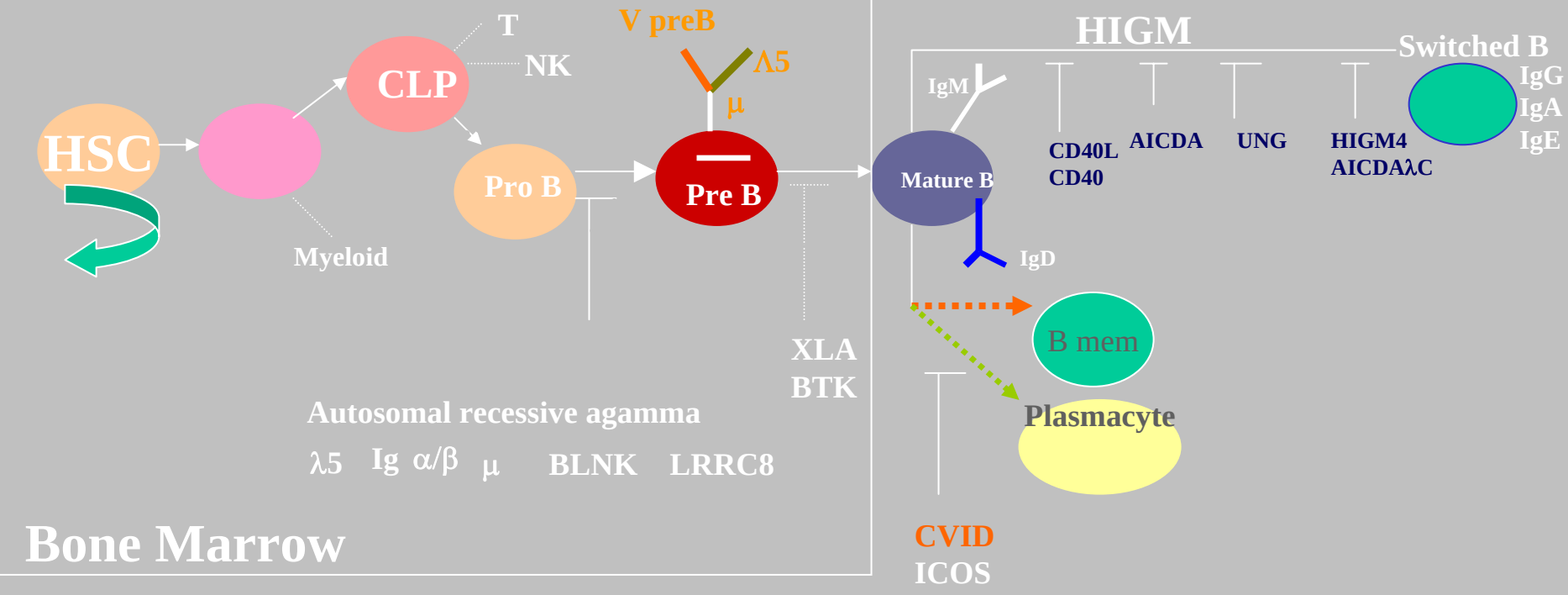
Table 2. Predominantly antibody deficiencies

Associated designation	Serum Ig	Circulating B cells	Presumed pathogenesis	Inheritance	Associated features
1. X-linked agammaglobulinaemia	All isotypes decreased	Profoundly decreased	Mutations in *btk*	XL	Severe bacterial infections
2. Autosomal recessive agammaglobulinaemia	All isotypes decreased	Profoundly decreased	Mutations in μ Igα, Igβ, $\lambda 5$, Vpreβ genes; or BLNK and syk genes	AR	Severe bacterial infections
3. Ig heavy-chain gene deletions	IgG1 or IgG2, IgG4 absent and in some cases IgE and IgA1 or IgA2 absent	Normal or decreased	Chromosomal deletion at 14q32	AR	Not always symptomatic
4. κ Chain deficiency mutations at AR	Ig(K) decreased: antibody response normal or decreased	Normal or decreased κ-bearing cells	Point mutations at chromosome 2p11 in some patients	AR	–
5. Selective Ig deficiency					
(a) IgG subclass deficiency	Decrease in one or more IgG isotypes	Normal or immature	Defects of isotype differentiation	Unknown	Not always symptomatic
(b) IgA deficiency	Decrease in IgA1 and IgA2	Normal or decreased sIgA+	Failure of terminal differentiation in IgA+ve B cells	Variable	Autoimmune or allergic disorders; some have infections
6. Antibody deficiency with normal or elevated Igs	Normal	Normal	Unknown	Unknown	Selective inability to make antibody to polysaccharides
7. Common variable immunodeficiency	Decrease in IgG and usually IgA, ± IgM	Normal or decreased	Variable; undetermined	Variable	See below[a]
8. Transient hypogammaglobulinaemia of infancy	IgG and IgA decreased	Normal	Differentiation defect: delayed maturation of helper function	Unknown	Frequent in families with other Ids
9. AID deficiency	IgG and IgA decreased	Normal	Mutation in activation-induced cytidine deaminase gene	AR	Enlarged lymph nodes and germinal centres

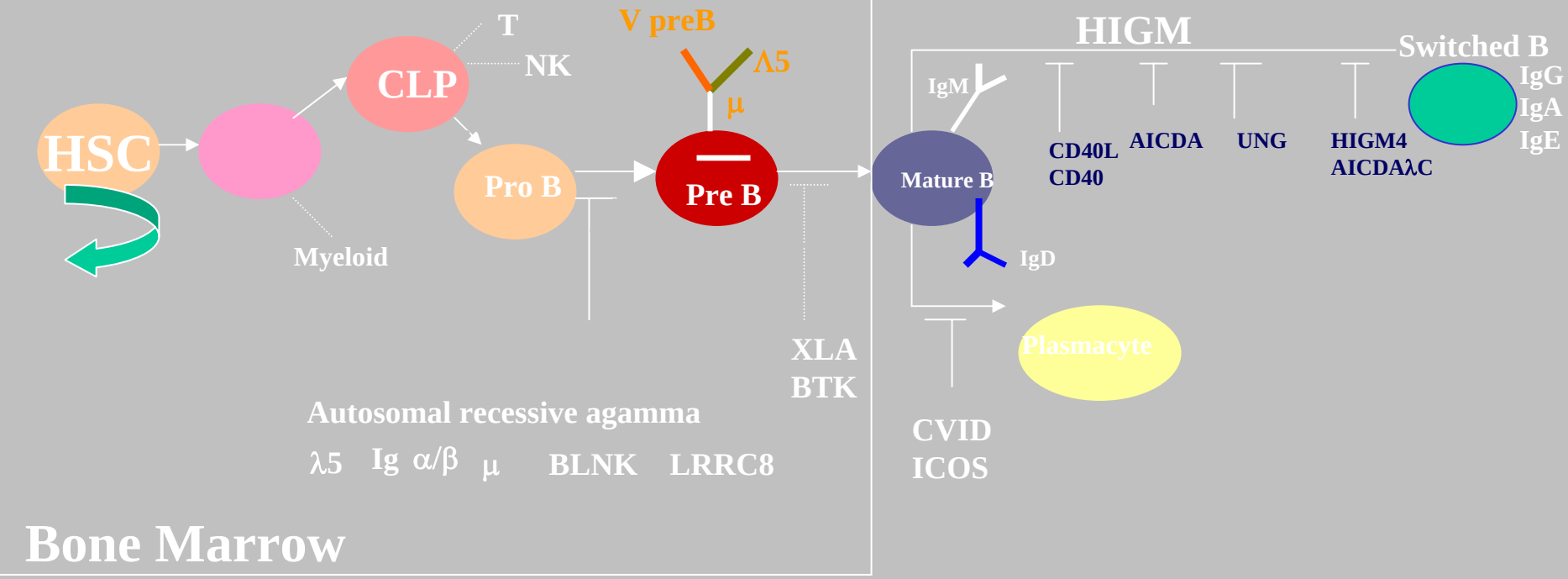
New defect: A deficiency of activation induced cytidine deaminse (AID) presents as a form of the hyper-IgM syndrome but differs from CD40L and CD40 deficiencies in that the patients have large lymph nodes with germinal centres and are not susceptible to opportunistic infections.

[a] Common variable immunodeficiency: there are several different clinical phenotypes, probably representing distinguishable diseases with differing immunopathogeneses.

Abbreviations: As for Table 1; Ig(K), immunoglobulin of kappa light-chain type; *btk*, Bruton's tyrosine kinase gene.



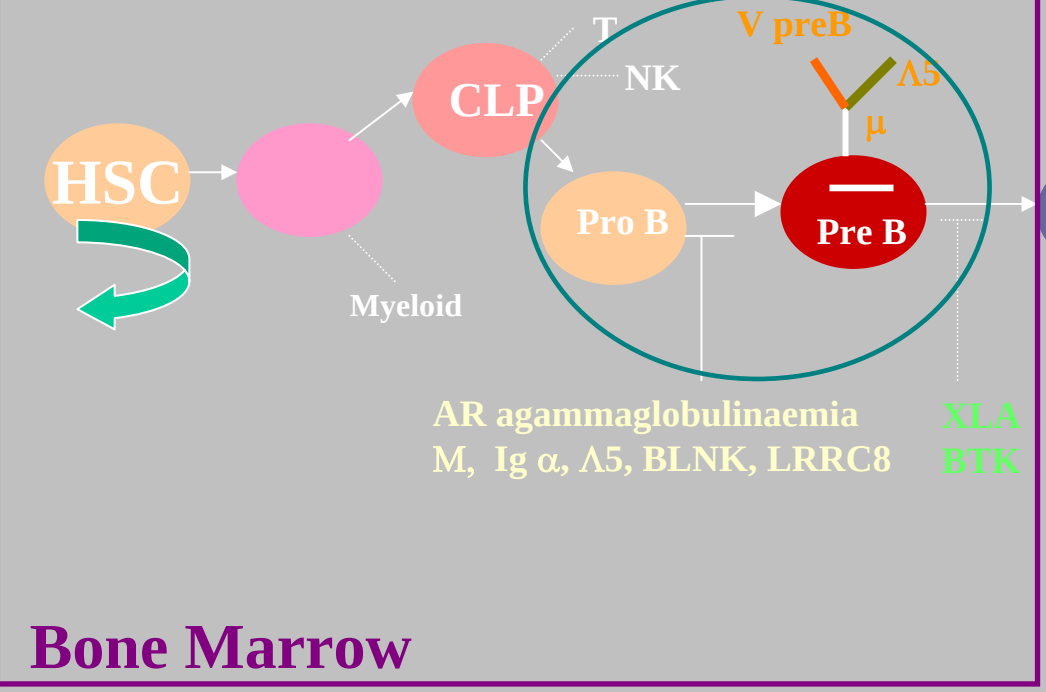
Adapted from : A Fischer Nature Immunology 2004

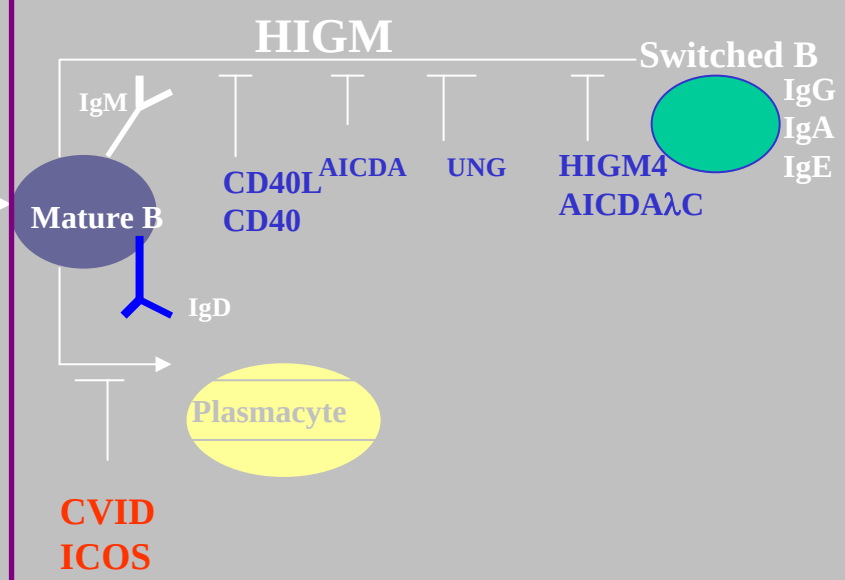


CD34, CD5, CD10, CD19, CD20 ….

IgG, IgA or IgE, CD38 , CD27 CD148

Adapted from : A Fischer Nature Immunology 2004





Bone Marrow

Immunoglobulin Production								
	IgM	0	0-low	Low	N-hi	N-hi	N-hi	N-hi
	Mutated IgM	0	0-low	Low	Low	0	biased	N
	IgG	0	0-low	Low	0	0	0-low	0-low
	IgA	0	0-low	Low	0	0	0	0-low

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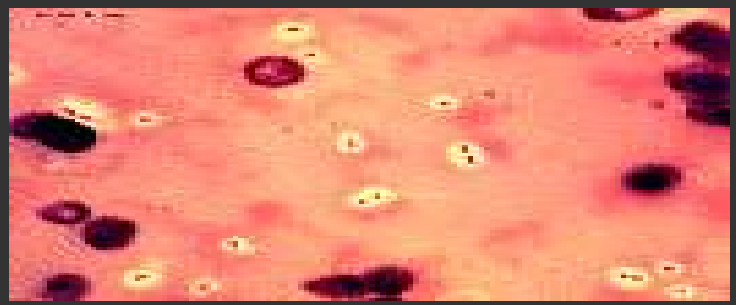
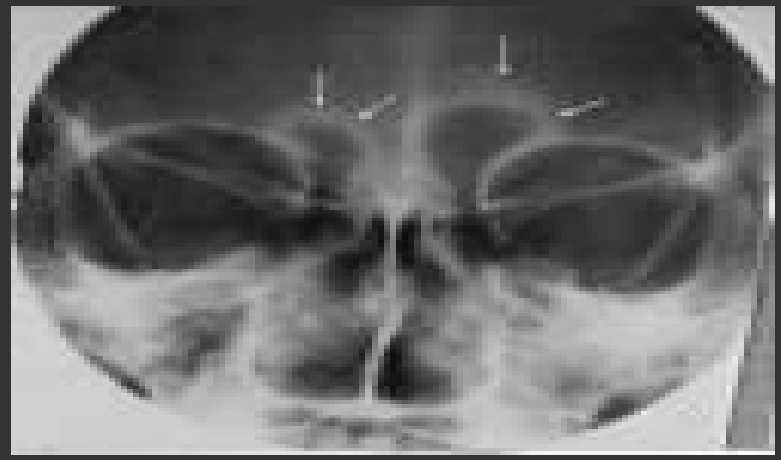
Susceptibility to encapsulated Bacteria

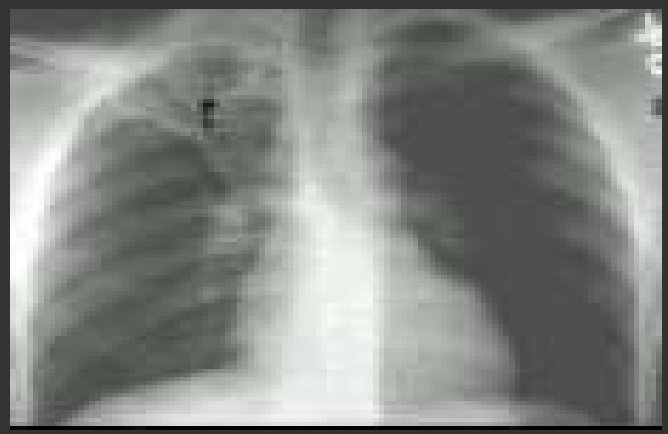
- H Influenzae



- S pneumoniae

Sinusitis





Pneumonia

Otitis Media

CVID

- Frequent, Bacterial Respiratory Infections,
- Chronic lung disease, Bronchiectasis is common

- Gastrointestinal , nodular lymphoid hyperplasia
- Splenomegaly
- Malignancies
- Autoimmune phenomena : AIHA, ITP

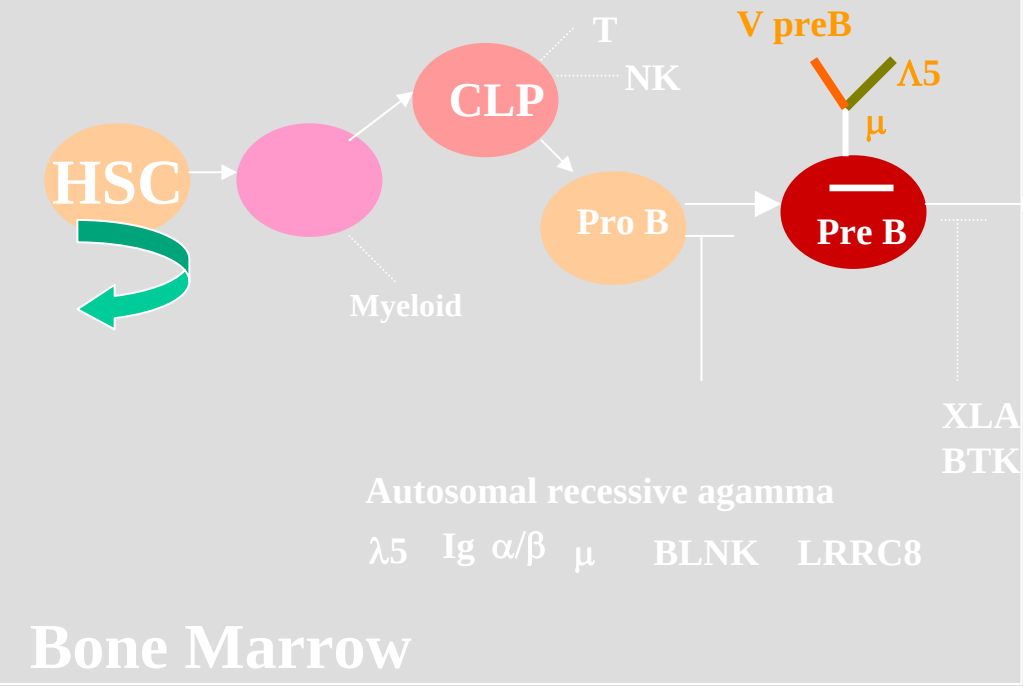
Criteria for CVID

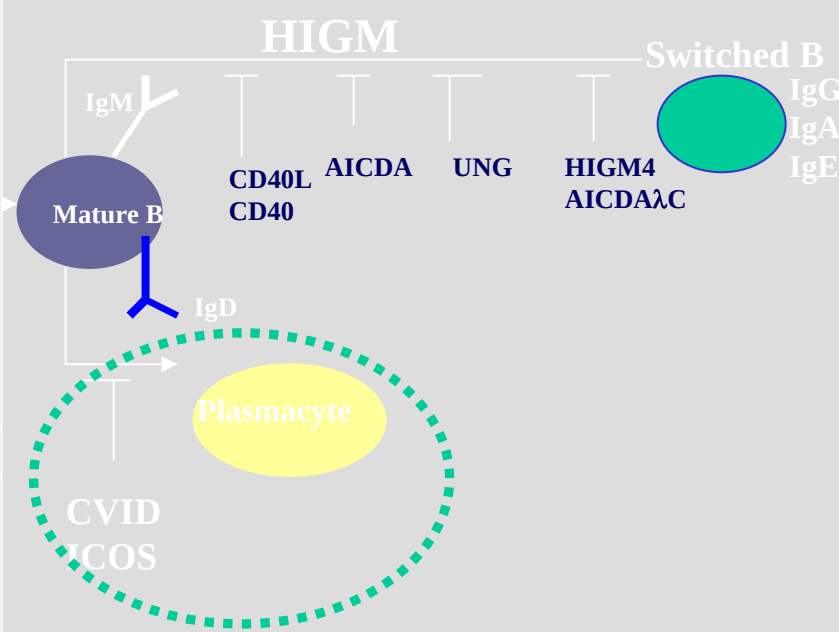
- Male /female
- > 2 years
- Poor responses to vaccines
- Serum IgG and IgA are > 2 SD below mean for age
- Exclude other 2nd Ab deficiencies

Spectrum of CVID

- **Estimated incidence 1 in 50,0000**
- **Aetiology unknown (multiple)**

- 2nd/3rd/4th decades of life
- Serum IgM can be normal in 50%
- Abnormalities in T cells occur in 30-40% cases





Immunoglobulin Production	IgM	0	0-low	Low	N-hi	N-hi	N-hi	N-hi
	Mutated IgM	0	0-low	Low	Low	0	biased	N
	IgG	0	0-low	Low	0	0	0-low	0-low
	IgA	0	0-low	Low	0	0	0	0-low

Adapted from : A Fischer Nature Immunology 2004

Genetics of CVID

Various inheritance patterns
AR, AD, X-linked
Sporadic cases – most common

Linked to MHC and IgAD
ICOS (Grimbacher et al)

Search for CVID candidate proteins, 4/32 patients lacked ICOS, the "inducible costimulator" on activated T cells, due to an inherited homozygous deletion in the ICOS gene.
T cells normal: subset distribution, activation, cytokine production and proliferation**.** **BUT** naive, switched and memory B cells were reduced.
Phenotype of human ICOS deficiency, suggests critical involvement of ICOS in T cell help for late B cell differentiation, class-switching and memory B cell generation

Classification of CVID

- Farrants method

- Took PBLs from CVID , kept cells alive for 1 week in the lab & got them to produce immunoglobulin
- **(the only cells that can make Ig are B cells,….. memory B cells)**

- He found he could divide CVID patients into 3 groups depending on the isotype of Ig they made.

Farrants Groups

- **Group A**
- **Don’t make any Immunoglobulin in vitro**

- **Group B**
- **Make IgM only**

- **Group C**
- **Make IgM, IgG & IgA (but have low serum levels)**

- **Normal healthy donors**
- **Make IgM, IgG & IgA (have normal serum levels)**

Farants method was time consuming & difficult to do.

While: Group A patients correlated with granulomatous disease & splenomegaly

- This method was not adopted generally

CVID Classification Cont'd

Recent reports described reduced populations of $CD27^+$ memory B cells and increased percentages of undifferentiated B cells in CVID blood.

This work has prompted 2 attempts to classify CVID based on rapid flow cytometric quantification of blood memory B cells and immature B cells.

JC Brouet, A Chedeville, JP Fermand and B Royer. Study of the B cell memory compartment in common variable immunodeficiency. Eur J Immunol 30 (2000) 2516-2520

S Jacquot, L Macon-Lemaitre, E Paris et al. B cell co-receptors regulating T cell dependant antibody production in common variable immunodeficiency: CD27 pathway defects identify subsets of severely immunocompromised patients Int Immunol 13 (2001) 871-876

Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, Eibel H, Schlesier M, Peter HH. Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar 1;99(5):1544-51.

Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, Debre P, Schmitt C, Oksenhendler E. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003 Sep;23(5):385-400

Carsetti R, Rosado MM, Donnanno S, Guazzi V, Soresina A, Meini A, Plebani A, Aiuti F, Quinti I. The loss of IgM memory B cells correlates with clinical disease in common variable immunodeficiency. J Allergy Clin Immunol. 2005 Feb;115(2):412-7.

POTENTIAL NEW TYPE OF CLASSIFICATION FOR CVID

- Based on peripheral blood
- Especially B lymphocytes – producers of immunoglobulin
- Using antibodies to identify different types of B lymphocytes
- Look at numbers of memory B lymphocytes

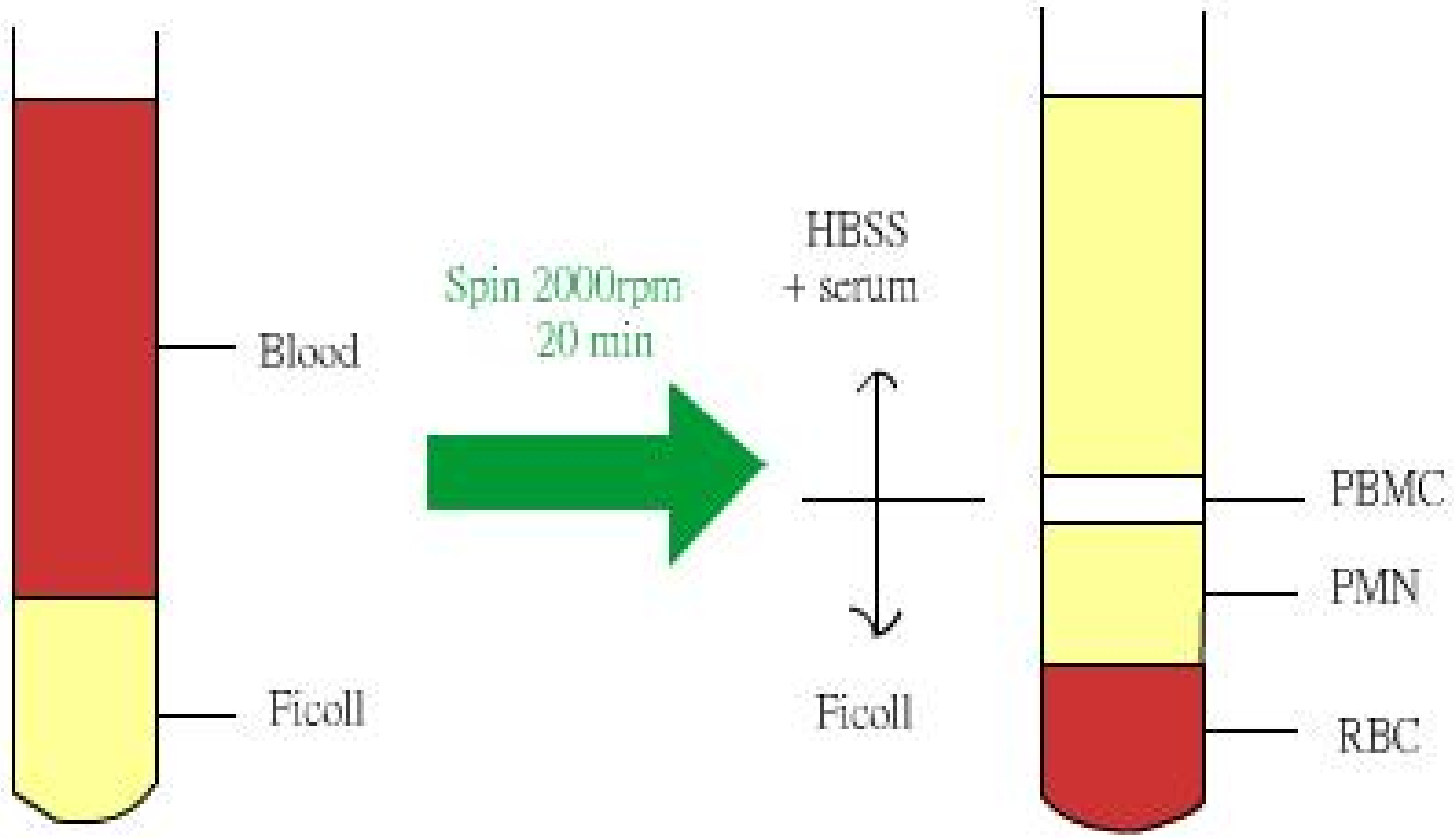
Memory B Cells

- B cells make up 6-10% of all PBLs in healthy person
- Memory B cells make up approx **1.6%** of all PBLs in healthy person
- BUT Memory B cells appear to make much lower in CVID patients
- Make up < 0.4% of all PBLs in some CVID patients.

Classification cont’d

- **The production of immunoglobulin *(in lab*) seems to be dependent on the presence of memory B cells**

Methods to examine B memory lymphocytes

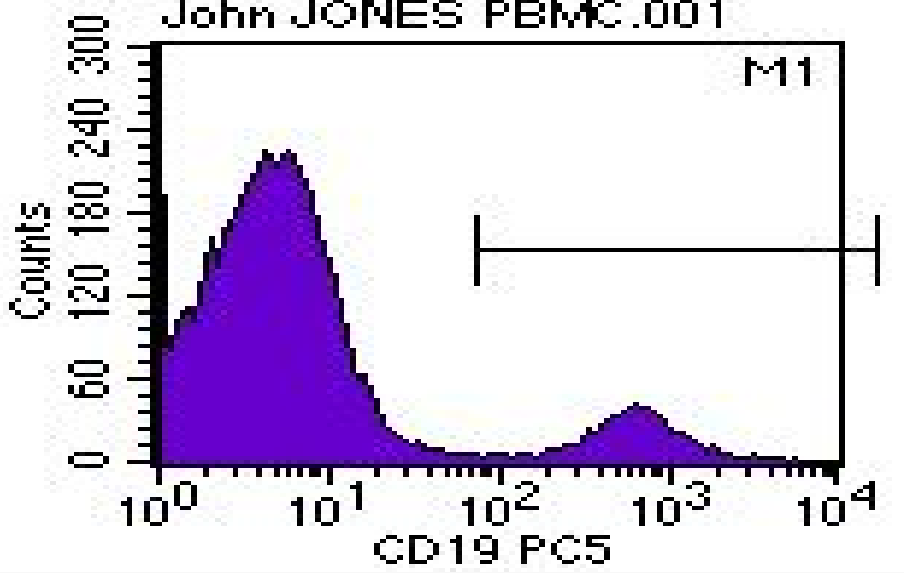


•PBLs, contain different types of B cells, including Memory B cells (also T & NK cells)

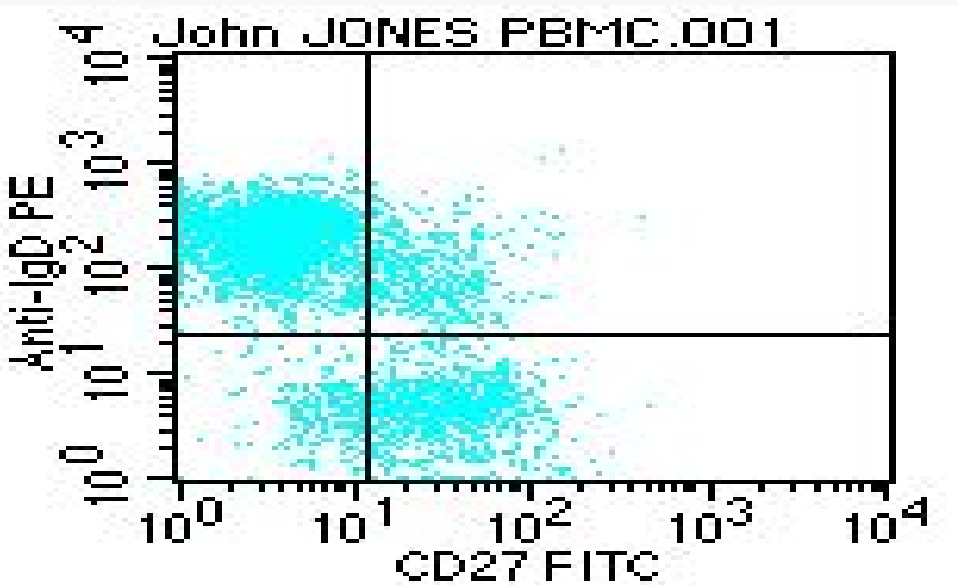
Methods Cont'd

- Add antibodies that define memory B cells to the PBLs

- Antibodies are
- Anti-CD27
- Anti-IgM
- Anti-IgD
- Anti-CD19



GATE on CD19 B cells



Examine CD27 positive and negative and IgD expression on CD19 positive B cells

Classification A

Warnatz K, Denz A, Drager R, Braun M, Groth C, Wolff-Vorbeck G, Eibel H, Schlesier M, Peter HH.

Severe deficiency of switched memory B cells (CD27(+)IgM(-)IgD(-)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. Blood. 2002 Mar 1;99(5):1544-51.

Warnatz et al 2002

Class-switched CD27(+) IgD(-) memory B cells

- < 0.4% of PBLs in 77% CVID (group I)
- > 0.5% of PBLs in 23% of CVID patients (group II).

- 0.5% in all healthy donors
- Correlates with IgG production *in vitro*

- Group Ia > 20% $CD21^+$ B cells
- Group Ib < 20% CD21+ B cells

Classification B

Piqueras B, Lavenu-Bombled C, Galicier L, Bergeron-van der Cruyssen F, Mouthon L, Chevret S, Debre P, Schmitt C, Oksenhendler E.

Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. J Clin Immunol. 2003 Sep;23(5):385-400

Piqueras et al 2003

Group **MB2** (19%) with normal memory B cells

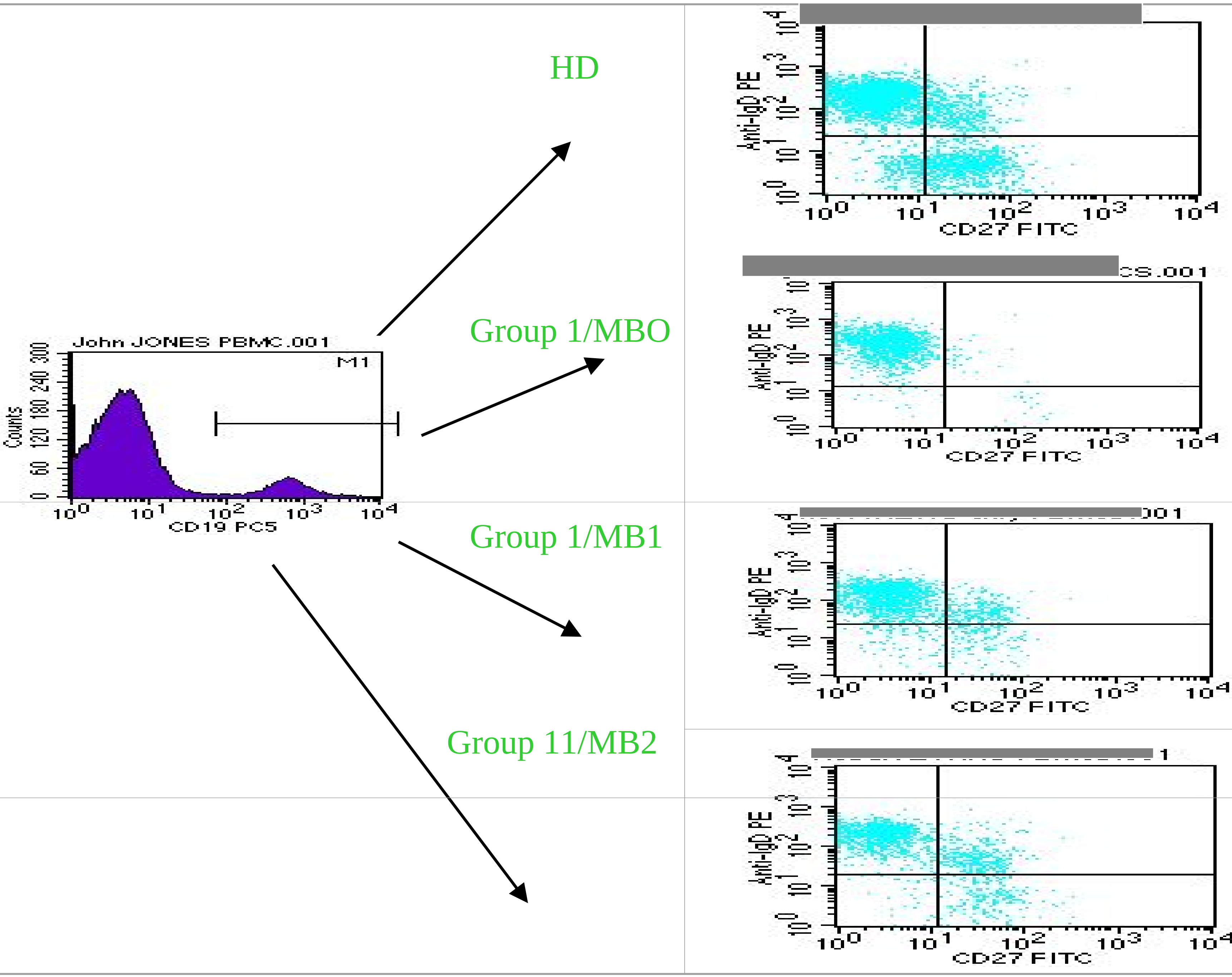
Group **MB1** (33%) defective switched (IgD-CD27+)

normal non switched (IgD+CD27+)

Group **MB0** (47%) Almost no memory B cells.

MB0/MB1 = Group I

Piqueras *Warnatz*



HD
Group 1/MBO
Group 1/MB1
Group 11/MB2
John JONES PBMC.001
M1
Counts
CD19 PC5
Anti-IgD PE
CD27 FITC
CS.001
001
1

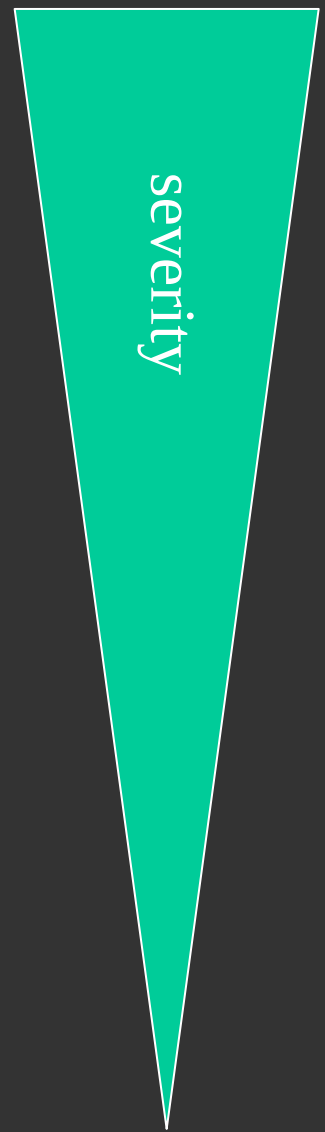
Both classifications correlate with some clinical aspects

Higher prevalence in MB0/MB1/ Group 1

Splenomegaly:	(59%)	(*42%) in MB1*
Lymphoid proliferation	(48%)	
Granulomatous disease	(44%)	

Classification of CVID

Bryant (Farant), 1990	**Warantz, 2002**	**Piqueras, 2003**
A no Ig production in vitro	Ia < 0.4% Class-switched CD27(+)IgM(-)IgD(-) memory B cells of PBLs > 20% $CD21^+$ of B cells	MB0 Almost no memory B cells
B IgM only	Ib < 0.4% Class-switched CD27(+)IgM(-)IgD(-) memory B cells of PBLs < 20% CD21+ B cells	MB1 defective switched (IgD-CD27+) normal non switched (IgD+CD27+)
C **low** serum levels of IgM, IgG, IgA	II > 0.5% CD27(+)IgM(-)IgD(-) of PBLs	MB2 normal memory B cells



Note

Although useful, probably all of these classification systems need to be re-analysed and patient diagnoses **reassessed** in the light of recent genetically identified immunodeficiencies, many of which had been previously categorized as CVID.

i.e XLP, ICOS and AID mutations.

Classifications depend on:

1. Defining tight cut-offs
2. Demonstrating immune phenotypes are stable with time *and not 2⁰ to complications of CVI including inter-current infections*.
3. Large collaborative studies needed.
4. Quality assurance of assays will play an important role **IF** classifications are to be used predicatively
5. Essential methods involved are **simple and reliable.**

For the classification to be useful in routine diagnosis, it is important that the flow cytometric method can be used **without** prior separation of peripheral blood mononuclear cells (PBMC).

Whole Blood method is now used.

1. Examined **23** CVID patients and **24** controls, using both PBMC and whole blood.
2. Excellent correlation between these methods.
3. Method was reproducible.
4. Classified CVID patients by all 3 existing classifications, including secretion of immunoglobulin by B cells *in vitro*

Staining programme: Preparation of Whole blood

1/ Add 500ul of whole blood (WB) to an LP4 tube and mark its volume with a marker

2/ Add 1.5mls of PBS then vortex and spin at 1000RPM for 5 mins.

3/ Aspirate the supernatant and then re-suspend in 1.5mls of PBS.

4/ Spin at 1000RPM for 5 mins.

5/ Repeat steps 3 and 4.

6/ Aspirate for a final time and fill the tube back up to the line with PBS.

Preparation of PBMC's

1/ After preparation of PBMC's gain a stock concentration of 5 x 10^6 cells/ml.

Once the whole blood and PBMC preparations are ready the cells can be stained.

Cell staining

Prepare a cocktail of the 4 antibodies being used and gently mix, for example;

TABLE 1 Characteristics of CVID Patients and Controls

No	Sex	Age	Age Onset	Age Diag	CD19 %	Lymph's mm^2	IgM/D+27- naive % PBL	IgM/D+27- naive % B	IgM/D+27+ IgD mem % PBL	IgM/D+27+ IgDmem % B	IgM/D-27+ Switched % PBL	IgM/D-27+ Switched % B	CD27+ % B	CD21-ve % B	Bryant	Warnatz PBMC	Warnatz WB	Piqueras PBMC	Piqueras WB
1	M	53	27	28	7.4	2399	7.30	98.50	0.10	1.30	0.003	0.04	1.34	18.20	A	1b	1b	MBO	MBO
2	M	73	63	71	4.0	702	3.8	96.3	0.13	3.3	0.01	0.22	3.52	1.94	A	1b	1b	MBO	MBO
3	M	67	62	63	1.7	2155	1.5	85	0.17	9.8	0.03	1.7	11.5	10.9	A	1b	1b	MB1	MB1
4	F	20	13	14	5.3	1282	4.8	91	0.1	2.2	0.03	0.5	2.7	2.02	A	1b	1b	MBO	MBO
5	M	68	65	65	4.2	n.d	3.8	89.5	0.2	4	0.10	3.2	7.2	64.7	A	1a	1a	MBO	MBO
6	F	50	39	41	30.4	746	29.6	82	0.3	8.7	0.10	2.5	11.2	20.4	A	1a	1b	MBO	MBO
7	M	55	35	35	5.3	730	4.9	92.4	0.2	4.7	0.10	1.5	6.2	35.07	A	1a	1a	MB0	MBO
8	M	40	33	36	2.2	1828	1.7	76.1	0.2	7.63	0.10	5.61	13.2	24.3	A	1a	1b	MB0	MB1
9	F	76	60	60	6.0	637	5.2	86.5	0.3	5.37	0.10	1.94	7.3	16.6	A	1b	1b	MB0	MBO
10	F	50	2	39	1.1	559	0.9	65.7	0.1	8.57	0.10	2.9	11.5	32.14	A	1a	1a	MB1	MB1
11	F	33	31	32	3.6	2940	2.6	71.7	0.9	24.6	0.10	2.3	26.9	3.49	A	1b	1b	MB1	MB1
12	M	18	16	16	19.0	1039	16.7	88.3	1.9	9.7	0.20	1.3	11	1.74	C	1b	1b	MB1	MB1
13	F	71	71	71	8.3	1468	5	60.6	2.6	31.5	0.37	4.64	36.1	39.83	B	1a	1a	MB1	MB1
14	F	40	39	39	8.6	695	6.5	75.1	1.5	17.9	0.36	4.13	22.6	4.7	B	1b	1b	MB1	MB1
15	F	22	17	17	9.9	769	7.7	79	1.3	13.6	0.28	3.61	17.2	17.8	A	1b	1a	MB1	MB1
16	F	54	43	49	11.2	1378	8.4	75	2.3	21	0.33	3.6	24.6	1.5	ND	1b	1b	MB1	MB1
17	F	72	64	66	12.2	917	8.8	71.9	1.6	12.7	0.36	2.96	16.7	21.88	B	1a	1a	MB1	MB1
18	F	25	2	6	6.0	1569	4.1	68.3	1.2	19	0.58	9.61	29.5	9.68	B	11	II	MB2	MB2
19	M	23	1	3	14.8	2011	11	73.9	2.8	19	0.70	4.82	23.8	7.65	C	11	II	MB1	MB1
20	M	39	38	38	4.7	2231	2.7	57.2	1.3	27.7	0.66	14.1	41.8	5.38	C	11	II	MB2	MB2
21	M	19	16	16	12.7	2054	9.6	75.6	1.3	10.5	1.37	10.75	21.5	1.86	A	11	II	MB2	MB2
22	M	44	13	26	6.3	1460	4	64.8	1.7	26.8	0.58	6.4	33.2	4.29	B	11	II	MB1	MB1
23	F	58	50	50	12.0	2041	6.7	65.5	1.1	19	0.60	9.8	28.8	56.71	B	11	1a	MB2	MB1

TABLE 2

	Sex	Age	Age Onset	Age Diag	CD19 %	Lymph' mm^2	IgM/D+27- naive % PBL	IgM/D+27- naive % B	IgM/D+27+ IgD mem % PBL	IgM/D+27+ IgD mem % B	IgM/D-27+ Switched % PBL	IgM/D-27+ Switched % B	CD27+ % B	CD21-ve % B
CVID														
Mean	**n = 23**													
PBMC	11M/12F	48	33	39	8.2 +/- 7 NS	1398	6.5 + / - 7 NS	78.9 +/- 11 NS	1 +/- 0.9 NS	12 +/- 9 NS	0.28 +/- 0.3 NS	2.62 +/- 3 NS	16.3 +/- 11 NS	16 +/- 17 NS
WB					8.7 +/- 8		7.1 +/ - 7	81.1 +/- 14	1.1 +/- 1	12 +/- 11	0.3 +/- 0.3	3.5 +/- 6	16.2 +/- 14	14.8 +/- 19
HD														
Mean	**n=24**													
PBMC	10M/14F	38			8.6 +/- 3	1892	5.6 +/- 2 NS	65 +/- 11 NS	1.2 +/- 0.5 NS	15 +/- 6 NS	1.4 +/- 0.5 NS	17.5 +/- 7 NS	29.8 +/- 8 NS	4.9 +/- 5 NS
WB					8.5 +/- 3		5.9 +/- 2	68.7 +/- 11	1.1 +/- 0.7	13.7 +/- 5	1.2 +/- 0.5	13 +/- 8	26.3 +/- 14	5.4 +/- 5

Correlation of Switched Memory cells: PBMC vs WB

Fig 1e
CVID: PBMC v WB CD27+/IgD- (as % B cells)

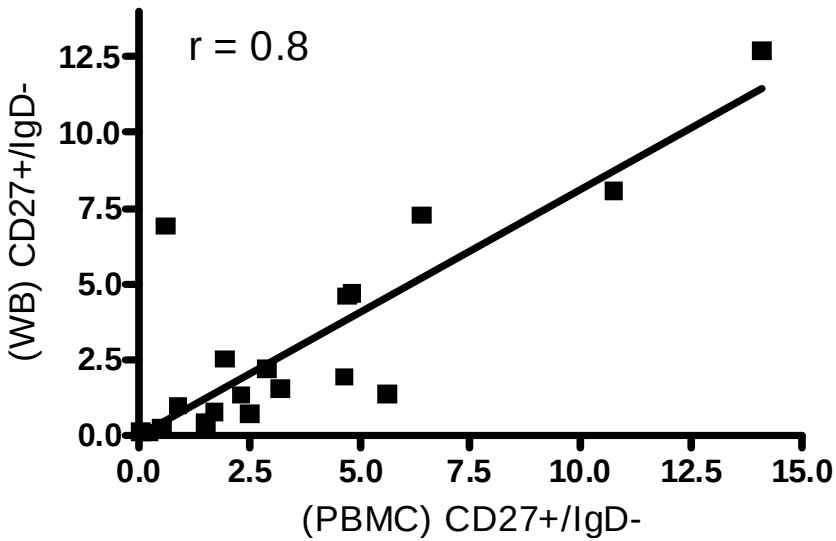
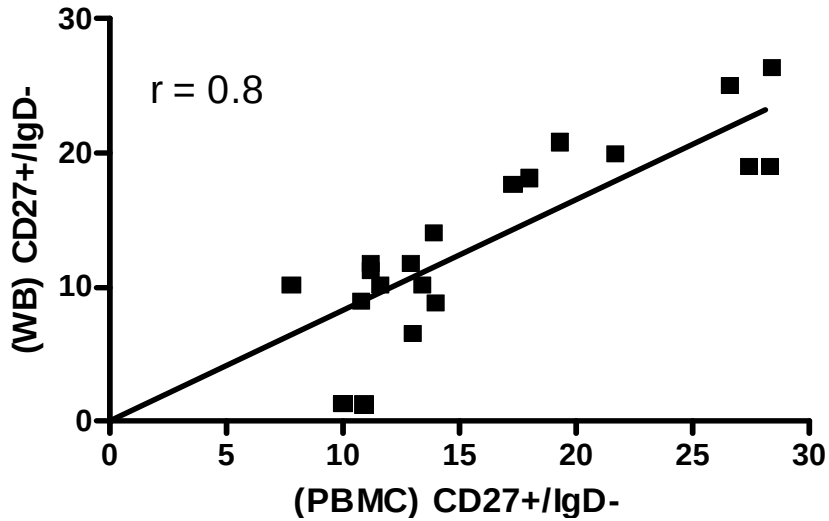


Fig 1f
HD:PBMC v WB CD27+/IgD- (as% B cells)



CVID

Healthy donors

Figure 1 Correlations of B cell subpopulations PBMC v WB

Fig 1a
CVID: PBMC V WB CD21- (as % B cells)

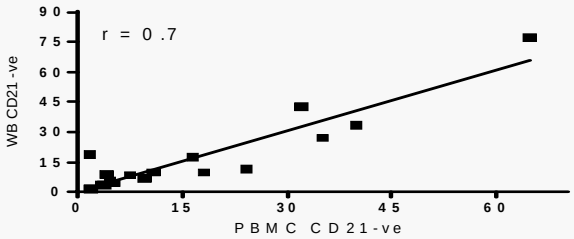


Fig 1b
HD: PBMC v WB CD21- (% of B cells)

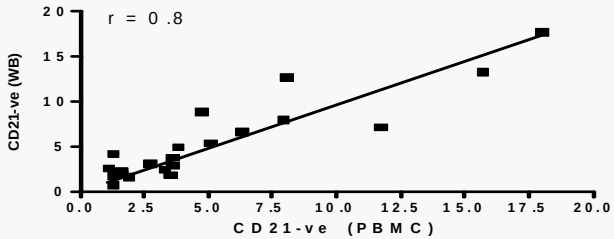


Fig 1c
CVID: PBMC v WB CD27+/IgD+ (as % B cells)

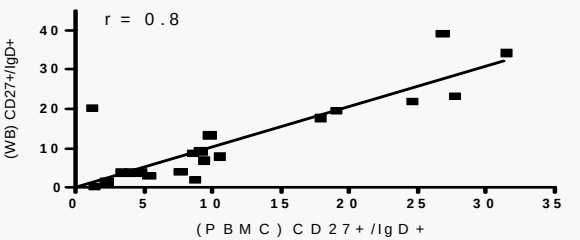


Fig 1d
HD:PBMC v WB CD27+/IgD+ (as % of B cells)

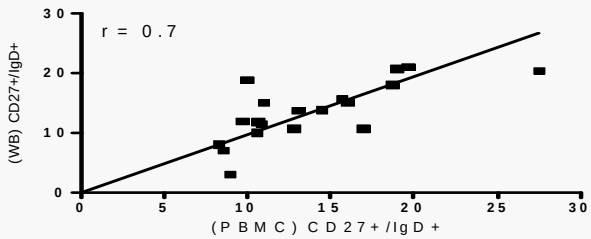


Fig 1e
CVID: PBMC v WB CD27+/IgD- (as % B cells)

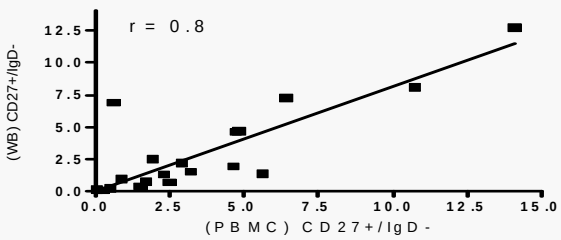


Fig 1f
HD:PBMC v WB CD27+/IgD- (as% B cells)

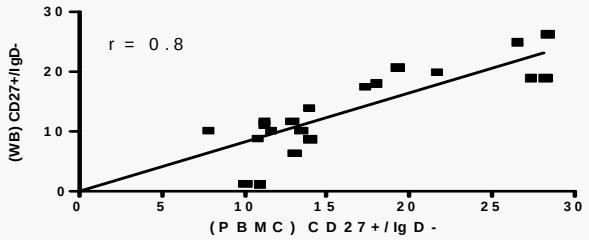


Fig 1g
CVID:PBMC v WB CD27+/IgD- (as % PBL)

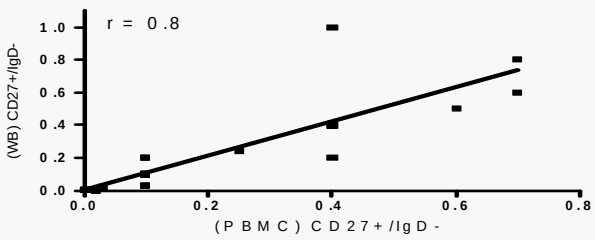
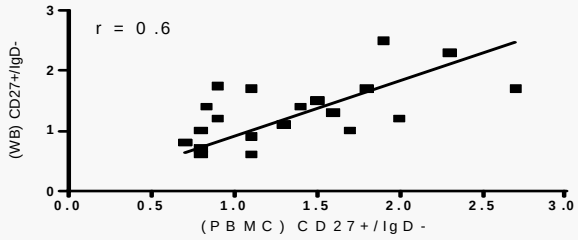
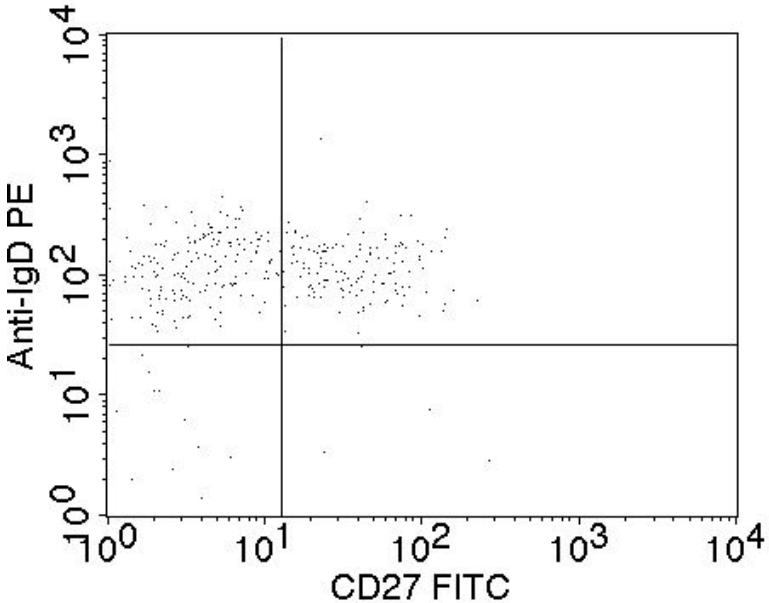


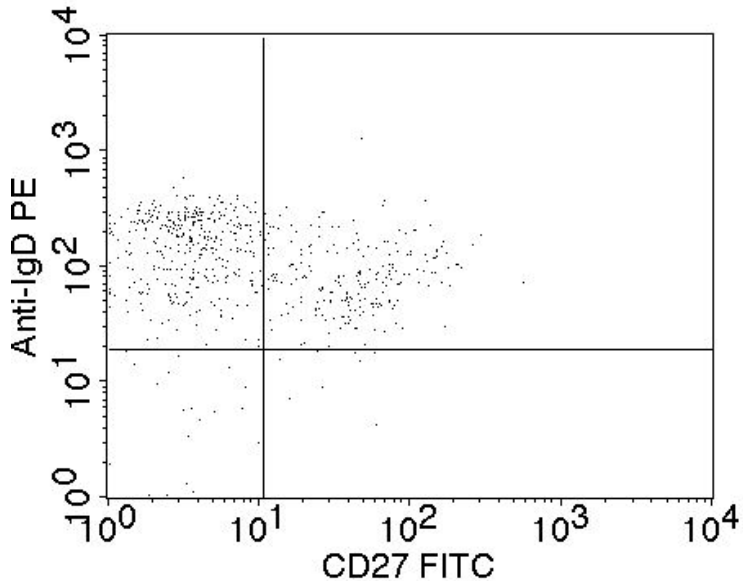
Fig 1h
HD:PBMC v WB CD27+/IgD- (as% PBL)

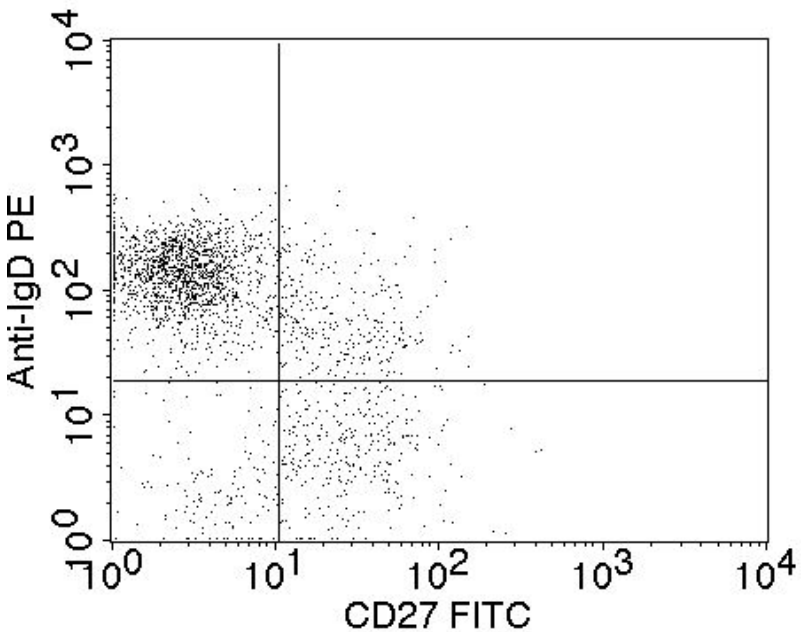


Pat 1: PBMC

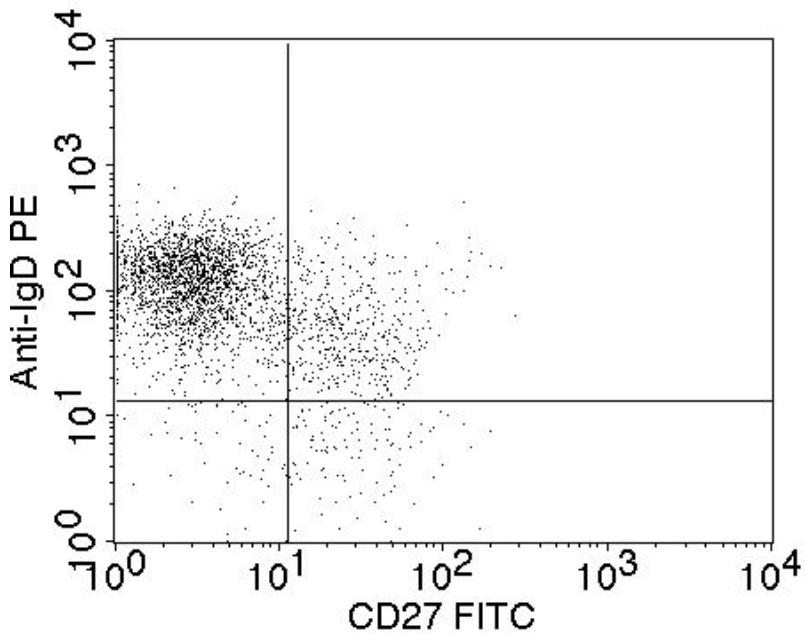


Pat 1: WB





Pat 2: PBMC



Pat 2: WB

Conclusions for WB Method

1. Fast, requires very little blood and is reproducible.

2. Percentages of naïve and memory cells from patients and controls were not significantly altered using WB or PBMC methods.

3. The WB method would ensure easy follow up of patients and allow monitoring of their memory B cell phenotype over time and in response to medications

Tube 1 cocktail

Antibody	Dilution*	Amount added to cocktail (ul)
CD27 FITC	1:5	20
IgD PE	1:5	20
CD19 PC5	neat	10
IgM Cy5	1:5	4

* All dilutions are in PBS

-

Once all of the cocktails have been prepared the cells can be stained

1/ 50ul of **PBMC's** + 10ul of cocktail

or 100ul of **WB** + 10ul of cocktail.

2/ Incubate for 15-30mins at 4°C in the dark.

3/ Add 500ul of FACS Lyse to the PBMC's and incubate for 5 mins.

Add 1.5mls of FACS Lyse to the WB and incubate for 5 mins.

4/ Centrifuge at 1200RPM for 5 mins.

5/ Decant supernatant and re-suspend in 300mls of PBS.

6/ Centrifuge at 1200RPM for 5 mins.

7/ Decant supernatant and re-suspend in 500ul of 1% formaldehyde.

Read on FACScalibur or store at 4°C overnight.

The WB described here is fast, requires very little blood and is reproducible. Percentages of naïve and memory cells obtained from patients and controls were not significantly altered using WB or PBMC methods. We confirm in this small cohort (Table 1) the previous finding that MB0/MB1 grouped together correlated with Warnatz group 1a and 1b, but not if MB0 and MB1 were used individually [Table 1 and Ref 13]. Warnatz et al have previously shown IgG production *in vitro* to be dependent on the presence of switched memory B cells [12]. In general, the findings from this small cohort of CVID patients would support this. 93% of Bryant Group A patients (no IgG, IgM or IgA production *in vitro*) were within the MB0/MB1 and Group 1 a/b categories, which have reduced numbers of switched and non- switched memory B cells. No Group B or C patient was found within the MBO category, which is the most severe of all the phenotypes defined by CD27 and IgD expression.

The WB method described in this report to quantitate CD27+ memory cells in peripheral blood would ensure easy follow up of patients and allow monitoring of their memory B cell phenotype over time and in response to medications

Other Questions

- What clinic days ?
- How many patients per day?
- What patients to begin with
- Liz Saxby & John Jones – staff involved.
- Berne can be main lab contact
- ? Would Zia coordinate?
- 20-30 mls EDTA blood
- 5 mls serum
- When to start?

FACScalibur Protocol for B cell markers

1/ Follow the normal turning on the FACS and calibrate both the LNW and LW. We will use the LW programme if possible.
2/ Go to research – B-cells – B cell acquisition.
3/ Get the browser via windows.
4/ Connect to the cytometer via acquire.
5/ Get counters up
6/ Create a new file – on the browser go to change (directory) – desktop – research - B cells – B cell controls – create a new file – select new file.
7/ Change file count to 1
8/ Change the patients name and the tube details accordingly
9/ Set the settings via Cytometer – instrument settings – desktop – Fascstation – Bd files – Instrument settings – calib file – set – done.
10/ Now set the voltages and compensation.
11/ THESE WILL NEED CHANGING FOR EACH TUBE. THE CORRECT SETTINGS ARE SHOWN BELOW.

	PBMC				WB			
Voltages	B1	B2	B3	B4	B1	B2	B3	B4
FSc	**2.00**	Same	Same	Same	**2.00**	Same	Same	Same
SSc	**417**	Same	Same	Same	**417**	Same	Same	Same
FL1	**574**	Same	**533**	**580**	**574**	**580**	**533**	**580**
FL2	**603**	Same	**594**	**603**	**603**	Same	**594**	**603**
FL3	**699**	Same	Same	Same	**699**	Same	Same	Same
FL4	**568**	Same	Same	Same	**549**	Same	**539**	**549**

Timing in the LAB

- **EDTA Blood 20-30 mls**
- 30 – 60 mins to get to lab
- 2-3 hours to separate cells

- Farrants
- 1-1.5 hour to set up Farrants method
- 7 days later ..ELISA takes 1 day

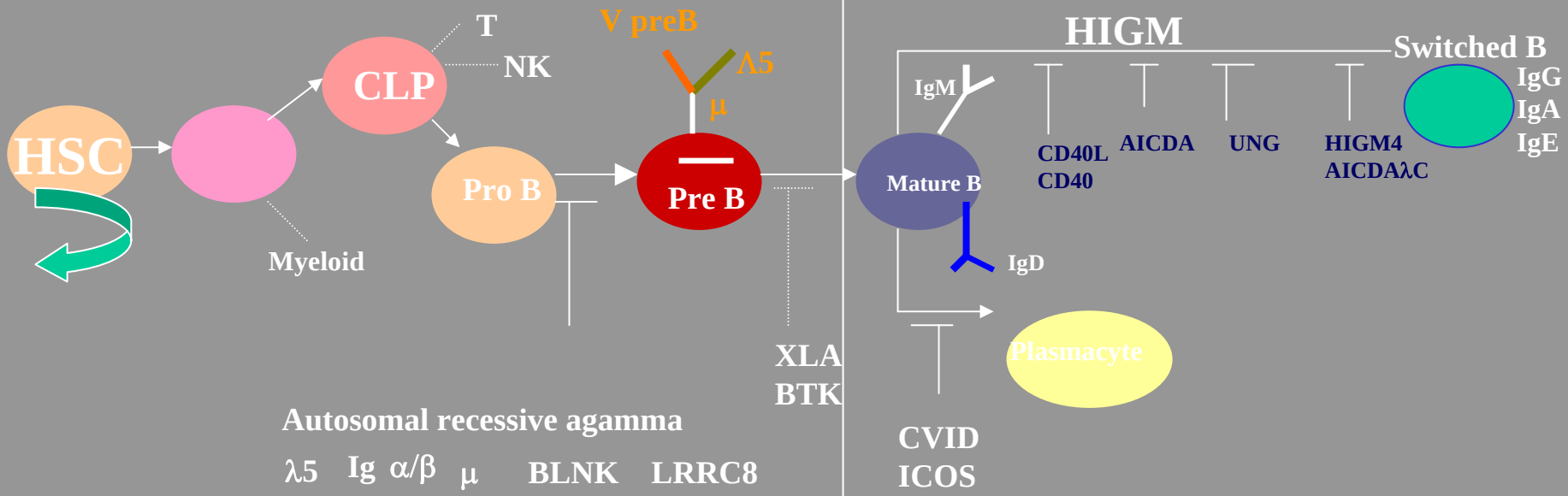
- **EDTA Blood 20-30 mls**
- 30-60 min to get to lab
- 2-3 hours to separate cells

- B Memory
- 1 hour to prepare antibodies 7 cells
- 1-2 hours to run on Flow
- Next day… interpret..2 hours

Heterogeneous Group of disorders

- Primary
  - **Single gene**
  - XLA
  - **Complex**
  - CVID
- Secondary
  - Malignancy
  - Renal/GI loss
  - Drugs

B cell immunodeficiencies as a result of defects in B cell development



Bone Marrow

Immunoglobulin Production	IgM	0	0-low	Low	N-hi	N-hi	N-hi	N-hi
	Mutated IgM	0	0-low	Low	Low	0	biased	N
	IgG	0	0-low	Low	0	0	0-low	0-low
	IgA	0	0-low	Low	0	0	0	0-low

Adapted from : A Fischer Nature Immunology 2004

Defects in Early B cell development

Recurrent Bacterial Infections

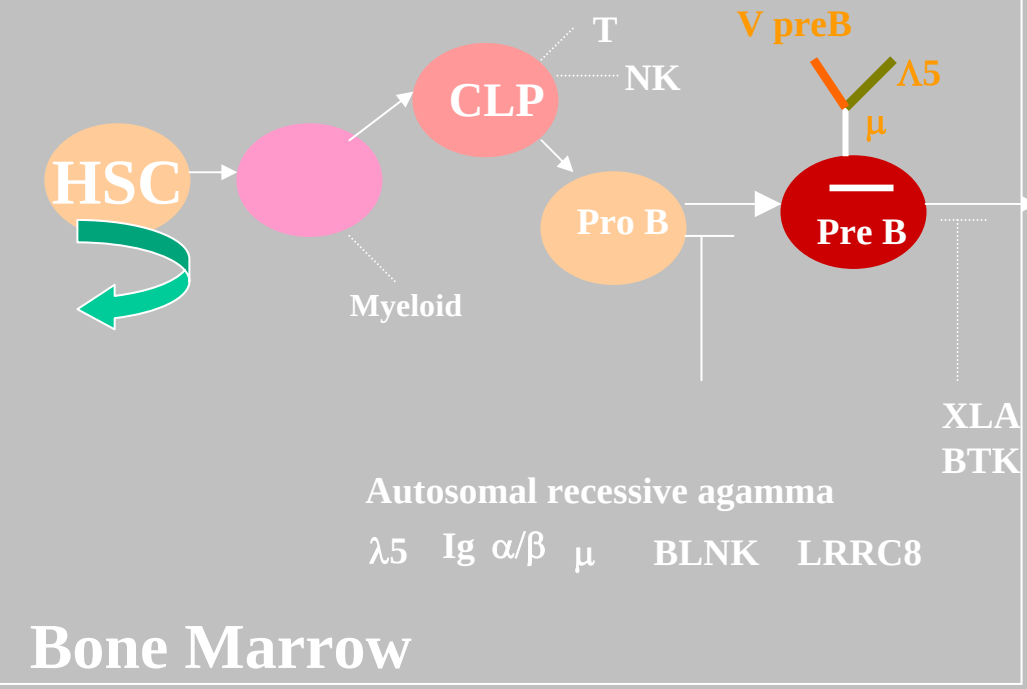
Hypogammaglobulinaemia

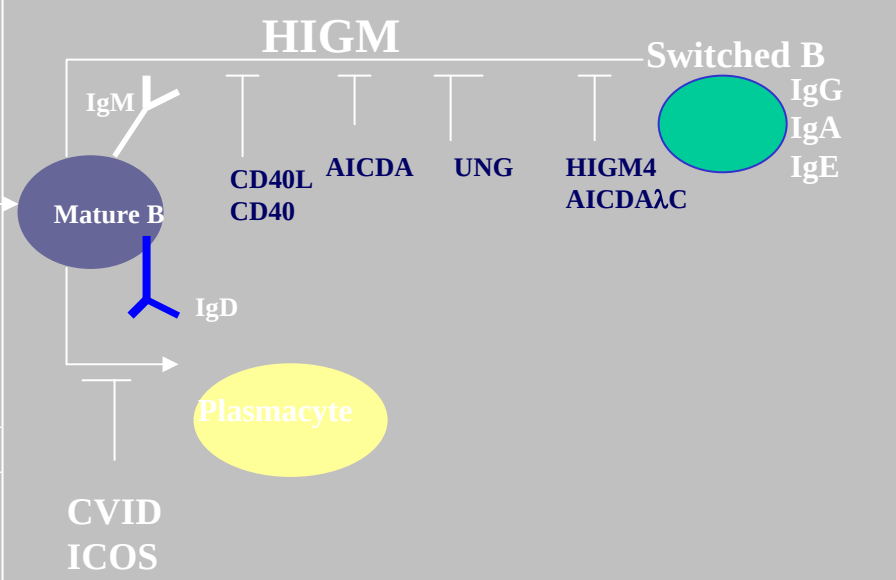
Reduced or Absent B cells

Paucity/ Absent tonsils

Hyper IgM Syndrome

Low/absent	**Serum IgG, IgA, IgE**
CSR	**Faulty**
Normal /elevated	**Serum IgM**
B cells in blood	**Normal**
65%	**Mutations in CD40L**
Others	**AID, UNG, CD40**





Immunoglobulin Production	IgM	0	0-low	Low	N-hi	N-hi	N-hi	N-hi
	Mutated IgM	0	0-low	Low	Low	0	biased	N
	IgG	0	0-low	Low	0	0	0-low	0-low
	IgA	0	0-low	Low	0	0	0	0-low

Adapted from : A Fischer Nature Immunology 2004

HIGM CD40L Deficiencies

Recurrent Bacterial

Opportunistic

30% pnemuocystis Carinii < 1 yr

Crytosporidium

Atypical mycobacteria

1. **Defects in CSR machinery.**

2. **Genetic linkage subset of HIGM**

3. **AICDA gene Activation Induced Cytidine DeAminase**

4. **AICDA: Expressed only in Germinal Centres**

5. **Enzyme: Activation induced deaminase AID.**

HIGM: AID

Patients did not undergo CSR: **but also**

Defects in generating somatic hypermutations. in Ig V segments

Linked CSR and SHM

Methods Cont'd

- We will also do Farrants method to compare.
- We hope to store DNA from the patients for future work (ethical permission would be needed). Look at T cell markers.
- Take serum, measure Igs, freeze serum,

Figure 4.19

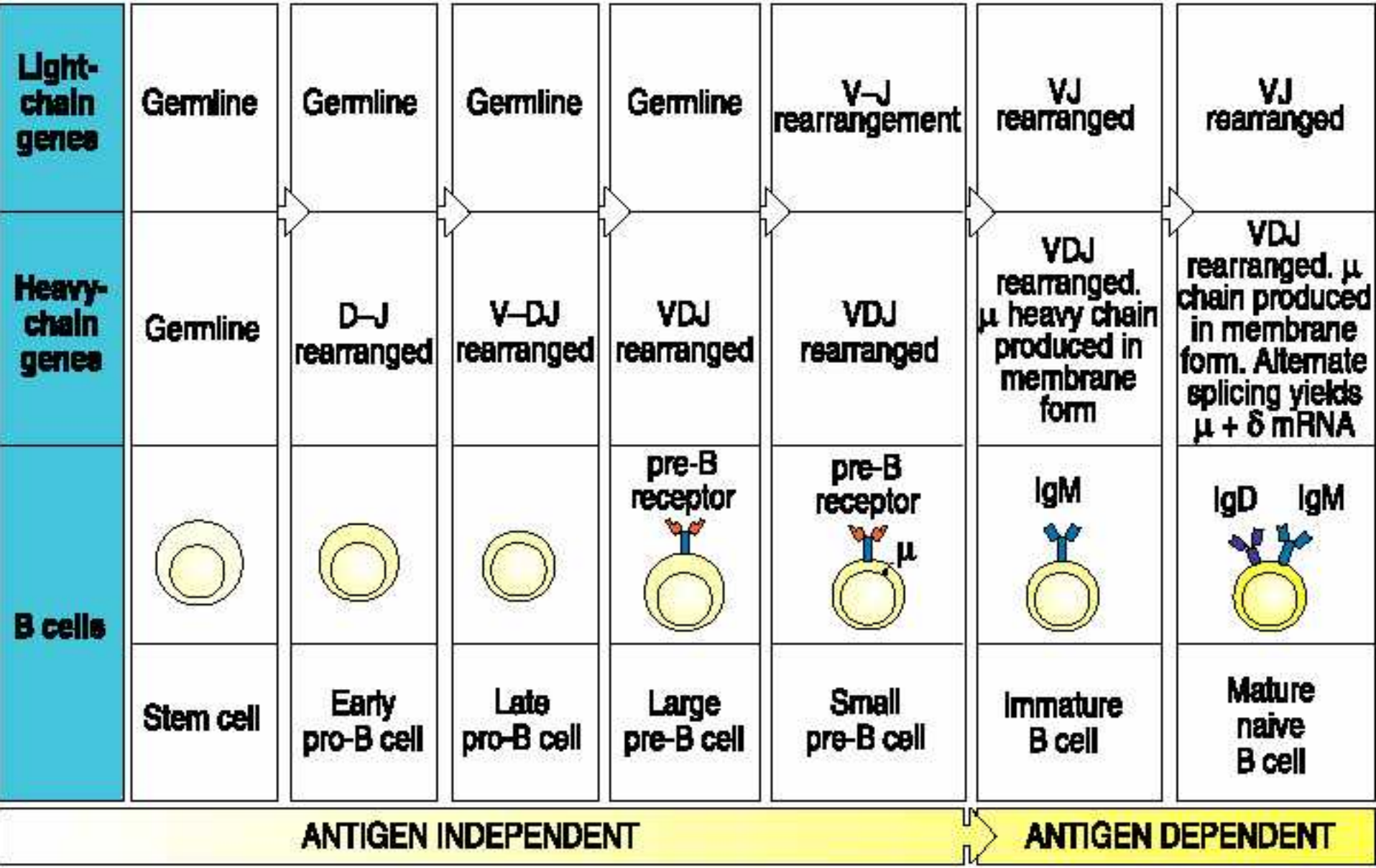


TABLE 2

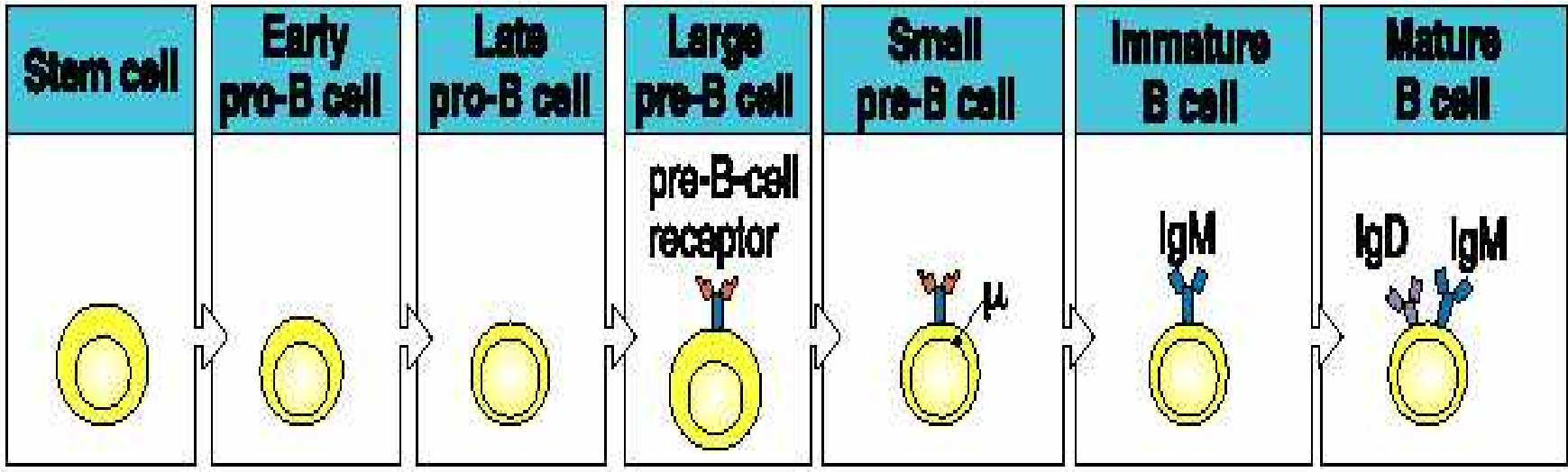
	Sex	Age	Age Onset	Age Diag	CD19 %	Lymph' mm^2	IgM/D+27- naive %PBL	IgM/D+27- naive %B	IgM/D+27+ IgD mem %PBL	IgM/D+27+ IgD mem %B	IgM/D-27+ Switched %PBL	IgM/D-27+ Switched %B	CD27+ %B	CD21-ve %B
Means	**CVID n = 23**													
PBMC	11M/12F	48	33	39	8.2 +/- 7 NS	1398	6.5 +/- 7 NS	78.9 +/- 11 NS	1 +/- 0.9 NS	12 +/- 9 NS	0.28 +/- 0.3 NS	2.62 +/- 3 NS	16.3 +/- 11 NS	16 +/- 17 NS
WB					8.7 +/- 8		7.1 +/- 7	81.1 +/- 14	1.1 +/- 1	12 +/- 11	0.3 +/- 0.3	3.5 +/- 6	16.2 +/- 14	14.8 +/- 19
Means	**HID n=24**													
PBMC	10M/14F	38			8.6 +/- 3	1892	5.6 +/- 2 NS	65 +/- 11 NS	1.2 +/- 0.5 NS	15 +/- 6 NS	1.4 +/- 0.5 NS	17.5 +/- 7 NS	29.8 +/- 8 NS	4.9 +/- 5 NS
WB					8.5 +/- 3		5.9 +/- 2	68.7 +/- 11	1.1 +/- 0.7	13.7 +/- 5	1.2 +/- 0.5	13 +/- 8	26.3 +/- 14	5.4 +/- 5

Patients and Healthy Donors

Ethical permission to study B cells in CVID patients and healthy donors (HD) was obtained from the Central Oxfordshire Research Ethics Committee.

Informed consent was obtained from 23 CVID patients who met the International (PAGID & ESID) diagnostic criteria; only those with normal numbers of circulating B cells were tested. CVID patients included those with granulomatous and autoimmune disease. Although not all CVID patients discussed in this report were tested individually for XLP, ICOS or HIGM mutations, their clinical histories and laboratory phenotypes strongly supported the CVID diagnosis. Amongst the Oxford patients, the mean duration of disease was 10.4 years (Range:newly diagnosed – 48 years) (24 HD were recruited from hospital staff. All patients were stable on immunoglobulin substitution and none were on additional medication. Blood samples from CVID patients were taken prior to Ig infusion.

Figure 4.3



	Stem cell	Early pro-B cell	Late pro-B cell	Large pre-B cell	Small pre-B cell	Immature B cell	Mature B cell
H-chain genes	Germline	D–J rearranged	V–DJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged	VDJ rearranged
L-chain genes	Germline	Germline	Germline	Germline	V–J rearrangement	VJ rearranged	VJ rearranged
Surface Ig	Absent	Absent	Absent	μ chain at surface as part of pre-B-cell receptor	μ chain in cytoplasm and at surface as part of pre-B-cell receptor	IgM expressed on cell surface	IgD and IgM made from alternatively spliced H-chain transcripts

TABLE 1 Characteristics of CVID Patients and Controls

No	Sex	Age	Age Onset	Age Diag	CD19 %	Lymph's mm^2	IgM/D+27- naive % PBL	IgM/D+27- naive % B	IgM/D+27+ IgD mem % PBL	IgM/D+27+ IgDmem % B	IgM/D-27+ Switched % PBL	IgM/D-27+ Switched % B	CD27+ % B	CD21-ve % B	Bryant	Warnatz PBMC	Warnatz WB	Piqueras PBMC	Piqueras WB
1	M	53	27	28	7.4	2399	7.30	98.50	0.10	1.30	0.003	0.04	1.34	18.20	A	1b	1b	MBO	MBO
2	M	73	63	71	4.0	702	3.8	96.3	0.13	3.3	0.01	0.22	3.52	1.94	A	1b	1b	MBO	MBO
3	M	67	62	63	1.7	2155	1.5	85	0.17	9.8	0.03	1.7	11.5	10.9	A	1b	1b	MB1	MB1
4	F	20	13	14	5.3	1282	4.8	91	0.1	2.2	0.03	0.5	2.7	2.02	A	1b	1b	MBO	MBO
5	M	68	65	65	4.2	n.d	3.8	89.5	0.2	4	0.10	3.2	7.2	64.7	A	1a	1a	MBO	MBO
6	F	50	39	41	30.4	746	29.6	82	0.3	8.7	0.10	2.5	11.2	20.4	A	1a	1b	MBO	MBO
7	M	55	35	35	5.3	730	4.9	92.4	0.2	4.7	0.10	1.5	6.2	35.07	A	1a	1a	MB0	MBO
8	M	40	33	36	2.2	1828	1.7	76.1	0.2	7.63	0.10	5.61	13.2	24.3	A	1a	1b	MB0	MB1
9	F	76	60	60	6.0	637	5.2	86.5	0.3	5.37	0.10	1.94	7.3	16.6	A	1b	1b	MB0	MBO
10	F	50	2	39	1.1	559	0.9	65.7	0.1	8.57	0.10	2.9	11.5	32.14	A	1a	1a	MB1	MB1
11	F	33	31	32	3.6	2940	2.6	71.7	0.9	24.6	0.10	2.3	26.9	3.49	A	1b	1b	MB1	MB1
12	M	18	16	16	19.0	1039	16.7	88.3	1.9	9.7	0.20	1.3	11	1.74	C	1b	1b	MB1	MB1
13	F	71	71	71	8.3	1468	5	60.6	2.6	31.5	0.37	4.64	36.1	39.83	B	1a	1a	MB1	MB1
14	F	40	39	39	8.6	695	6.5	75.1	1.5	17.9	0.36	4.13	22.6	4.7	B	1b	1b	MB1	MB1
15	F	22	17	17	9.9	769	7.7	79	1.3	13.6	0.28	3.61	17.2	17.8	A	1b	1a	MB1	MB1
16	F	54	43	49	11.2	1378	8.4	75	2.3	21	0.33	3.6	24.6	1.5	ND	1b	1b	MB1	MB1
17	F	72	64	66	12.2	917	8.8	71.9	1.6	12.7	0.36	2.96	16.7	21.88	B	1a	1a	MB1	MB1
18	F	25	2	6	6.0	1569	4.1	68.3	1.2	19	0.58	9.61	29.5	9.68	B	11	II	MB2	MB2
19	M	23	1	3	14.8	2011	11	73.9	2.8	19	0.70	4.82	23.8	7.65	C	11	II	MB1	MB1
20	M	39	38	38	4.7	2231	2.7	57.2	1.3	27.7	0.66	14.1	41.8	5.38	C	11	II	MB2	MB2
21	M	19	16	16	12.7	2054	9.6	75.6	1.3	10.5	1.37	10.75	21.5	1.86	A	11	II	MB2	MB2
22	M	44	13	26	6.3	1460	4	64.8	1.7	26.8	0.58	6.4	33.2	4.29	B	11	II	MB1	MB1
23	F	58	50	50	12.0	2041	6.7	65.5	1.1	19	0.60	9.8	28.8	56.71	B	11	1a	MB2	MB1