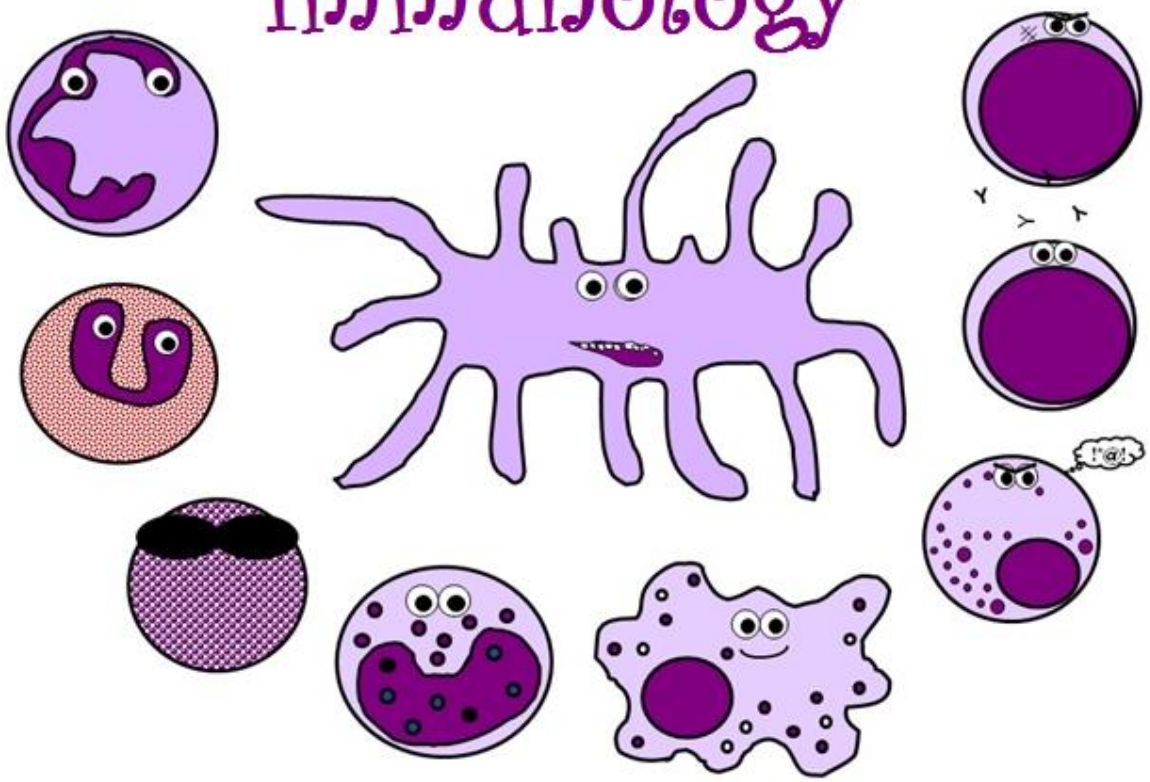
Immunology



Sheet Slides

Number: 9

Subject: Hyper IgM immunodeficiency

Done by: Omar Saffar

Corrected by: correction team

Doctor: Issa Abu Diyya

.....

.....

CASE 2 CD40 Ligand Deficiency

Failure of immunoglobulin class switching.

After exposure to an antigen, the first antibodies to appear are IgM. Later, antibodies of other classes appear: IgG predominates in the serum and extravascular space, while IgA is produced in the gut and in the respiratory tract, and IgE may also be produced in the mucosal tissues. The different effector functions of these different antibody classes are summarized in Fig. 2.1. The changes in the class of the antibody produced in the course of an immune response reflect the occurrence of heavy-chain isotype switching in the B cells that synthesize immunoglobulin, so that the heavy-chain variable (V) region, which determines the specificity of an antibody, becomes associated with

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	–	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Fig. 2.1 Each human immunoglobulin isotype has specialized functions and a unique distribution. The major effector functions of each isotype (+++) are shaded in dark red, while lesser functions (++) are shown in dark pink, and very minor functions (+) in pale pink. The distributions are similarly marked, with the actual average levels in serum shown in the bottom row. *IgG2 can act as an opsonin in the presence of Fc receptors of a particular allotype, found in about 50% of Caucasians.

Topics bearing on this case:

- Isotype or class switching
- Antibody isotypes and classes
- CD40 ligand and class switching
- Antibody-mediated bacterial killing

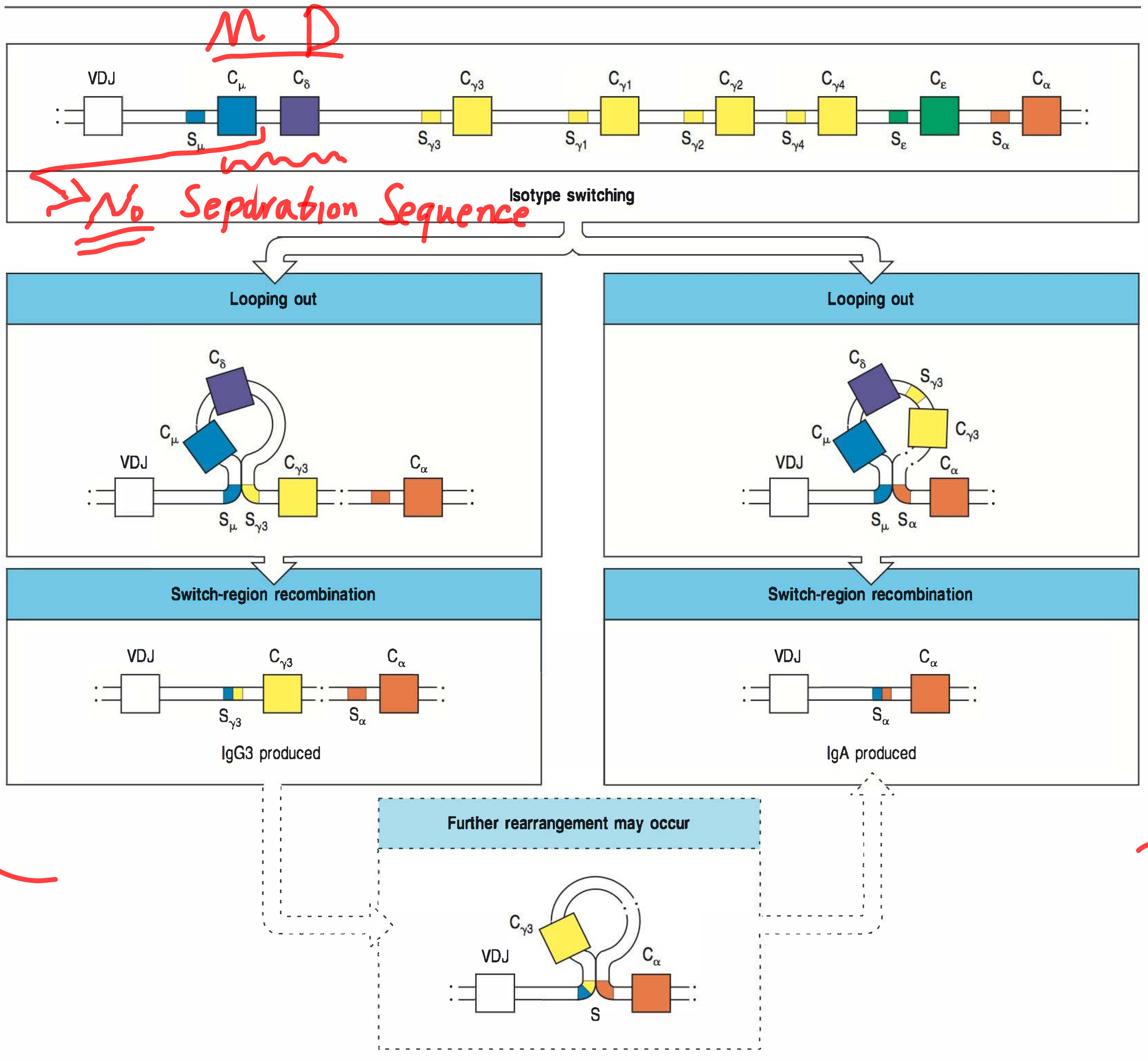


Fig. 2.2 Isotype switching involves recombination between specific switch signals. Repetitive DNA sequences that guide isotype switching are found upstream of each of the immunoglobulin C-region genes, with the exception of the C_δ gene. Switching occurs by recombination between these repetitive sequences or switch signals as a result of the repair of double-strand breaks (see Case 3), with deletion of the intervening DNA.The initial switching event takes place from the μ switch region (S_μ): switching to other isotypes can take place subsequently from the recombinant switch region formed after μ switching. S, switch region.

heavy-chain constant (C) regions of different isotypes, which determine the class of the antibody, as the immune response progresses (Fig. 2.2).

Class switching in B cells, also known as isotype switching and class-switch recombination, is induced mainly by T cells, although it can also be induced by T-cell independent Toll-like receptor (TLR)-mediated signaling. T cells are required to initiate B-cell responses to many antigens; the only exceptions are responses triggered by some microbial antigens or by certain antigens with repeating epitopes. This T-cell 'help' is delivered in the context of an antigen-specific interaction with the B cell (Fig. 2.3). The interaction activates the T cell to express the cell-surface protein CD40 ligand (CD40L, also known as CD154), which in turn delivers an activating signal to the B cell by binding CD40 on the B-cell surface. Activated T cells secrete cytokines, which are required at the initiation of the humoral immune response to drive the proliferation and differentiation of naive B cells, and are later required to induce class switching (Fig. 2.4). In humans, class switching to IgE synthesis is best understood, and is known to require interleukin-4 (IL-4) or IL-13, as well as stimulation of the B cell through CD40.

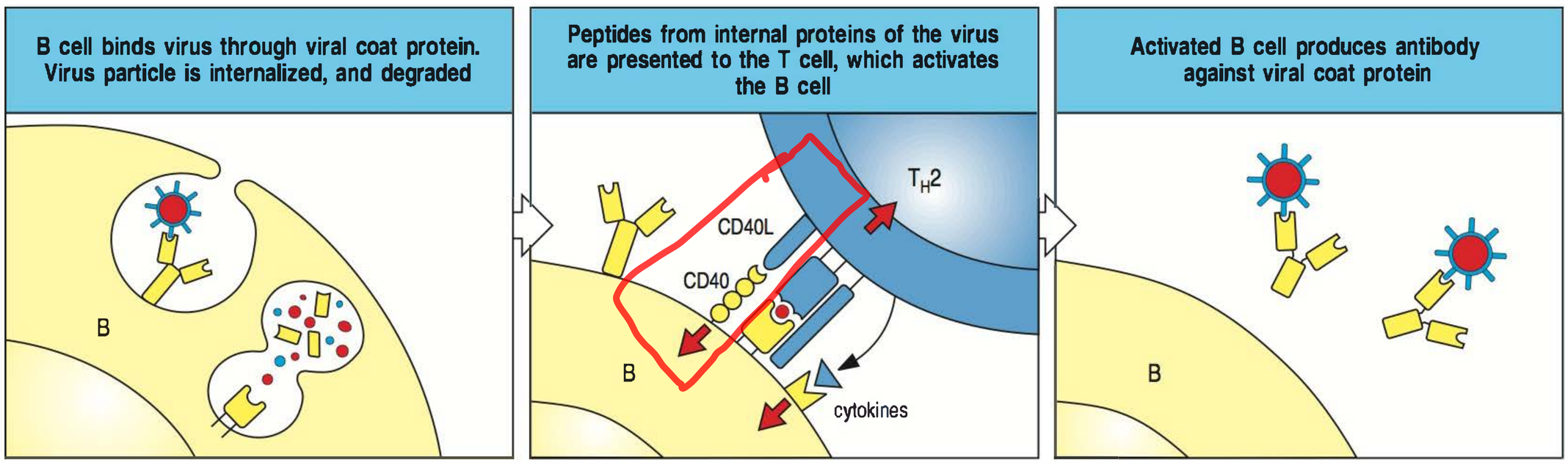


Fig. 2.3 B cells are activated by helper T cells that recognize antigenic peptide bound to class II molecules on their surface. An epitope on a viral coat (spike) protein is recognized by the surface immunoglobulin on a B cell, and the virus is internalized and degraded. Peptides derived from viral proteins are returned to the B-cell surface bound to MHC class II molecules, where they are recognized by previously activated helper T cells that activate the B cells to produce antibody against the virus.

The gene for CD40L (*CD40LG*) is located on the X chromosome at position Xq26. In males with a defect in this gene, isotype switching fails to occur; such individuals make only IgM and IgD and are severely impaired in their ability to switch to IgG, IgA, or IgE synthesis. This phenotype is known generally as 'hyper IgM syndrome,' and can also be due to defects other than the absence of CD40L (see Case 3). Similarly, defective class switching is also observed in patients with CD40 deficiency, a rare autosomal recessive condition. Defects in class switching can be mimicked in mice in which the genes for CD40 or CD40L have been disrupted by gene targeting; B cells in these animals fail to undergo switching. The underlying defect in patients with CD40L deficiency can be readily demonstrated by isolating their T cells and challenging them with soluble, fluorescently labeled CD40 (made by engineering the extracellular domain of CD40 onto the constant region (Fc) of IgG) or with monoclonal antibodies that recognize the CD40-binding epitope of CD40L. *In vitro* activated T cells from patients with CD40L deficiency fail to bind the soluble CD40–Fc (Fig. 2.5).

CD40 is expressed not only on B cells but also on the surfaces of macrophages, dendritic cells, follicular dendritic cells (FDCs), mast cells, and some epithelial and endothelial cells. Macrophages and dendritic cells are antigen-presenting

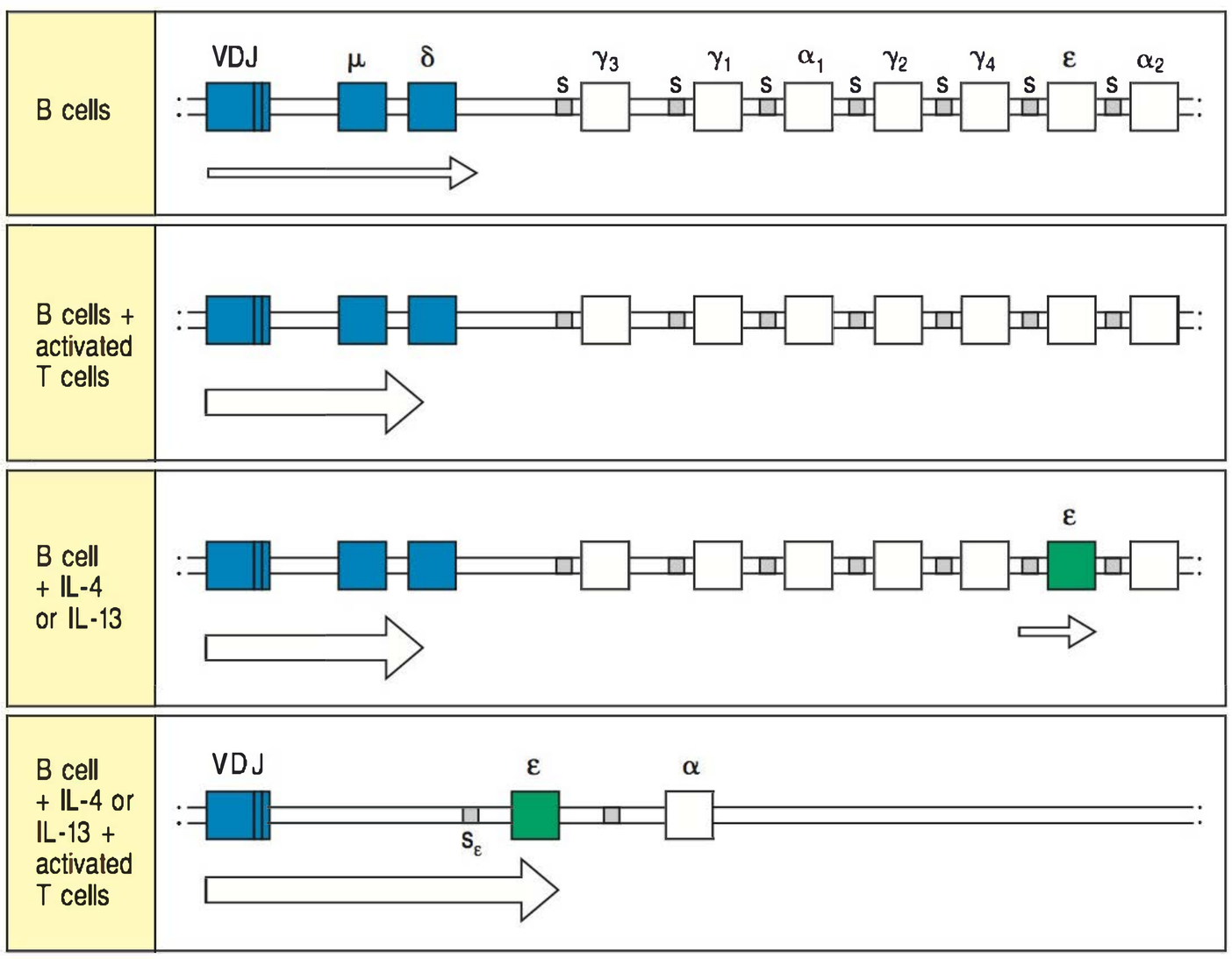


Fig. 2.4 Class switching to IgE production by human B cells. Purified human B cells in culture transcribe the μ and δ loci at a low rate, giving rise to surface IgM and IgD. On co-culture with T cells activated with ionomycin and phorbol myristate acetate (PMA), IgM is secreted. The presence of IL-4 or IL-13 stimulates an isotype switch to IgE. Purified B cells cultured alone with these cytokines transcribe the Cε gene at a low rate, but the transcripts originate in the switch region preceding the gene and do not code for protein. On co-culture with activated T cells in addition to IL-4 or IL-13, an isotype switch occurs, mature ε RNA is expressed, and IgE is synthesized.

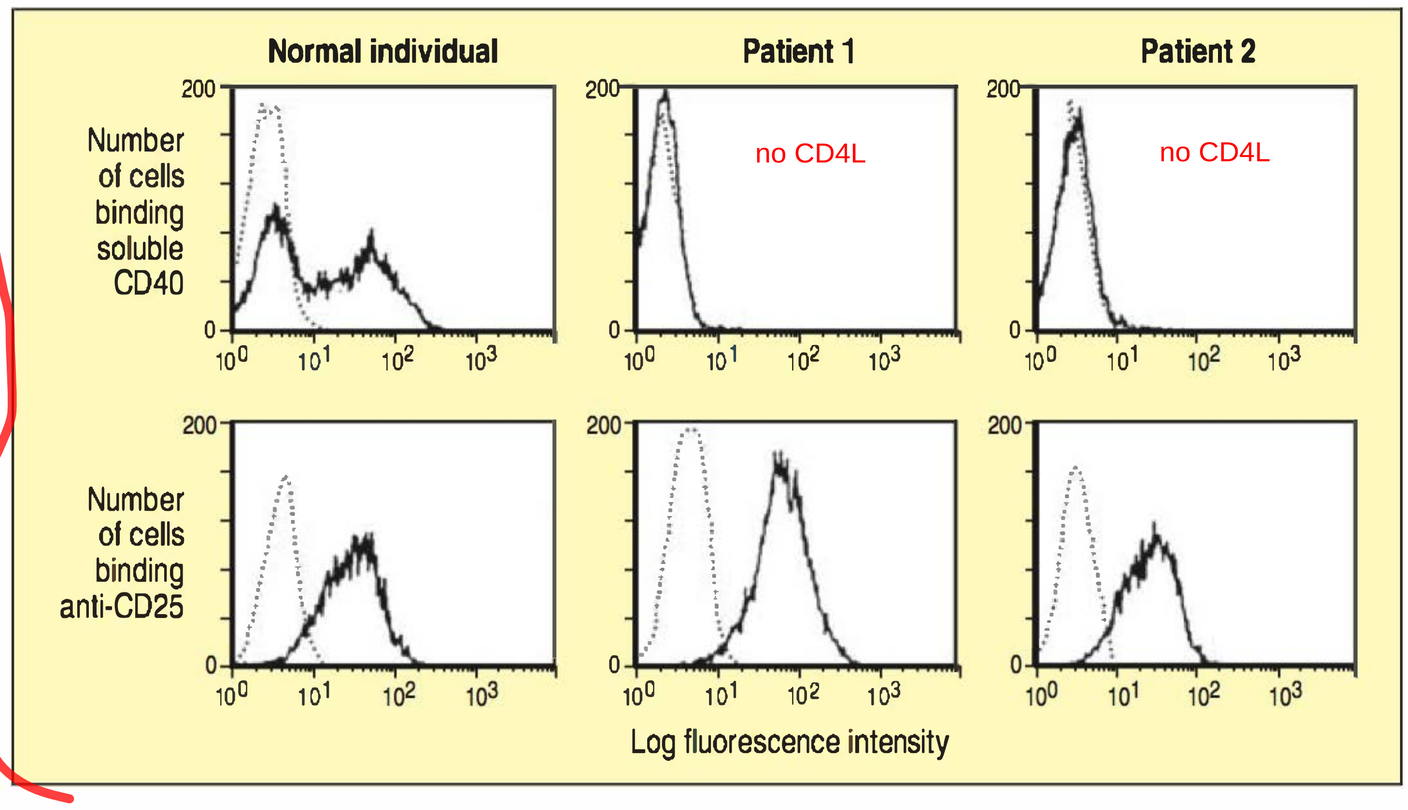


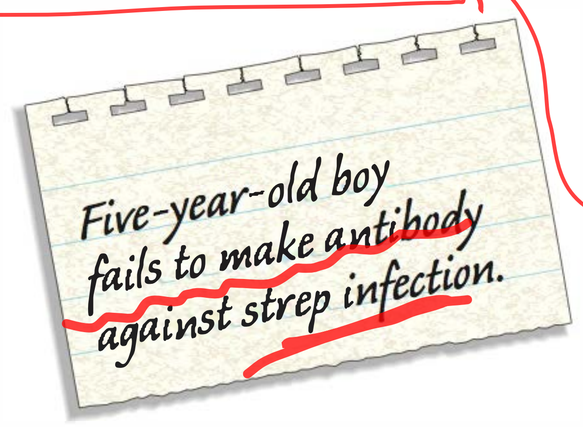
Fig. 2.5 Flow cytometric analysis showing that activated T cells from hyper IgM patients do not express the CD40 ligand. T cells from two patients and one healthy donor were activated *in vitro* with a T-cell mitogen, incubated with soluble CD40 protein, and analyzed by flow cytometry (see Fig. 1.3). The results are shown in the top three panels. In the normal individual, there are two populations of cells: one that does not bind CD40 (the peak to the left, with low-intensity fluorescence) and one that does (the peak to the right, with high-intensity fluorescence). The dotted line is the negative control, showing nonspecific binding of a fluorescently labeled protein to the same cells. In the patients with CD40L deficiency (center and right-hand panels), CD40 fluorescence exactly coincides with the nonspecific control, showing that there is no specific binding to CD40 by these cells. The bottom panels show that the T cells have been activated by the mitogen, because the T cells of both the normal individual and the two patients have increased expression of the IL-2 receptor (CD25), as expected after T-cell activation. The negative control is fluorescent goat anti-mouse immunoglobulin.

cells that can trigger the initial activation and expansion of antigen-specific T cells at the start of an immune response. Experiments in CD40L- or CD40-deficient patients and in gene-targeted CD40L-deficient mice indicate a role for the CD40–CD40L interaction in this early priming event, because in the absence of either CD40L or CD40 the initial activation and expansion of T cells in response to protein antigens is greatly reduced. The impairment of T-cell activation is the basis of some severe clinical features that distinguish CD40L and CD40 deficiency from other conditions characterized by a pure antibody deficiency.

The case of Dennis Fawcett: a failure of T-cell help.

pneumocystis carinii

it supposed to be higher because he have infections



Dennis Fawcett was 5 years old when he was referred to the Children's Hospital with a severe acute infection of the ethmoid sinuses (ethmoiditis). His mother reported that he had had recurrent sinus infections since he was 1 year old. Dennis had pneumonia from an infection with *Pneumocystis jirovecii* when he was 3 years old. These infections were treated successfully with antibiotics. While he was in the hospital with ethmoiditis, group A β-hemolytic streptococci were cultured from his nose and throat. The physicians caring for Dennis expected that he would have a brisk rise in his white blood cell count as a result of his severe bacterial infection, yet his white blood cell count was 4200 μl^{-1} (normal count 5000–9000 μl^{-1}). Sixteen percent of his white blood cells were neutrophils, 56% were lymphocytes, and 28% were monocytes. Thus his neutrophil number was low, whereas his lymphocyte number was normal and the number of monocytes was elevated.

Seven days after admission to the hospital, during which time he was successfully treated with intravenous antibiotics, his serum was tested for antibodies against streptolysin O, an antigen secreted by streptococci. When no antibodies against the streptococcal antigen were found, his serum immunoglobulins were measured. The IgG level was 25 mg dl^{-1} (normal 600–1500 mg dl^{-1}), IgA was undetectable (normal 150–225 mg dl^{-1}), and his IgM level was elevated at 210 mg dl^{-1} (normal 75–150 mg dl^{-1}). A lymph-node biopsy showed poorly organized structures with an absence of secondary follicles and germinal centers (Fig. 2.6).

Dennis was given a booster injection of diphtheria toxoid, pertussis antigens, and tetanus toxoid (DPT) as well as typhoid vaccine. After 14 days, no antibody was detected against tetanus toxoid or against typhoid O and H antigens. Dennis had red blood

No Reaction Against Specific Antigens!

cells of group O. People with type O red blood cells make antibodies against the A substance of type A red cells and antibodies against the B substance of type B red cells. This is because bacteria in the intestine have antigens that are closely related to A and B antigens. Dennis's anti-A titer was 1:3200 and his anti-B titer 1:800, both very elevated. His anti-A and anti-B antibodies were of the IgM class only.

His peripheral blood lymphocytes were examined by fluorescence-activated cell sorting analysis, and normal results were obtained: 11% reacted with an antibody against CD19 (a B-cell marker), 87% with anti-CD3 (a T-cell marker), and 2% with anti-CD56 (a marker for natural killer (NK) cells). However, all of his B cells ($CD19^+$) had surface IgM and IgD and none were found with surface IgG or IgA. When his T cells were activated *in vitro* with phorbol ester and ionomycin (a combination of potent polyclonal T-cell activators), they did not bind soluble CD40.

Dennis had an older brother and sister. They were both well. There was no family history of unusual susceptibility to infection.

Dennis was treated with intravenous gamma globulin, 600 mg kg^{-1} body weight each month, and subsequently remained free of infection until 15 years of age, when he developed severe, watery diarrhea. Cultures of the stools grew *Cryptosporidium parvum*. Within a few months, during which his diarrhea persisted in spite of treatment with the antibiotic azithromycin, he developed jaundice. His serum total bilirubin level was 8 mg dl^{-1}, and the serum level of conjugated bilirubin was 7 mg dl^{-1}. In addition, levels of γ-glutamyl transferase (γ-GT) and of the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated at 93 IU l^{-1}, 120 IU ml^{-1} and 95 IU ml^{-1}, respectively, suggesting cholestasis. A liver biopsy showed abnormalities of biliary ducts (vanishing bile ducts) that progressed to sclerosing cholangitis (chronic inflammation and fibrosis of the bile ducts). In spite of supportive treatment, Dennis died of liver failure at 21 years of age.

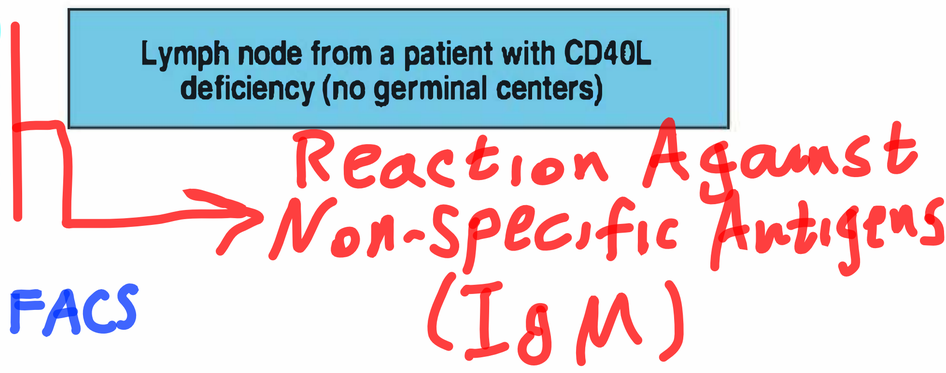


Fig. 2.6 Comparison of lymph nodes from a patient with CD40L deficiency (upper panel) and a normal individual (lower panel). Lower photograph courtesy of A. Perez-Atayde.

CD40 ligand deficiency (CD40L deficiency).

Males with a hereditary deficiency of CD40L exhibit consequences of a defect in both humoral and cell-mediated immunity. As we saw in Case 1, defects in antibody synthesis result in susceptibility to so-called pyogenic infections. These infections are caused by pyogenic (pus-forming) bacteria such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*, which are resistant to destruction by phagocytic cells unless they are coated (opsonized) with antibody and complement. On the other hand, defects in cellular immunity result in susceptibility to opportunistic infections. Bacteria, viruses, fungi, and protozoa that often reside in our bodies and only cause disease when cell-mediated immunity in the host is defective are said to cause opportunistic infections.

Dennis revealed susceptibility to both kinds of infection. His recurrent sinusitis, as we have seen, was caused by *Streptococcus pyogenes*, a pyogenic infection. He also had pneumonia caused by *Pneumocystis jirovecii* and diarrhea caused by *Cryptosporidium parvum*, a fungus and a protozoan, respectively, that are ubiquitous and cause opportunistic infections in individuals with defects in cell-mediated immunity.

Patients with a CD40L deficiency can make IgM in response to T-cell independent antigens but they are unable to make antibodies of any other isotype, and they cannot make antibodies against T-cell dependent antigens, which leaves the patient largely unprotected from many bacteria. They also have a defect in cell-mediated immunity that strongly suggests a role for CD40L in the T cell-mediated activation of macrophages. *Cryptosporidium* infection

humoral ↓ → pyogenic!
cellular ↓ → opportunistic!

macrophages need CD40L to binfd to it to activate it to secrete GM-CSF which induces proliferation of neutrophils! thats's why there was neutropenia!!

macrophages need CD40L to bind
it to activate it to secrete GM-CSF
which induces proliferation of
neutrophils!
thats's why there was neutropenia!

can cause persistent inflammation in the liver, and ultimately sclerosing cholangitis and liver failure. In addition, individuals with CD40L deficiency have severe neutropenia, with a block at the promyelocyte/myelocyte stage of differentiation in the bone marrow. Although the mechanisms underlying the neutropenia in these patients remain unclear, the lack of neutrophils accounts for the presence of severe sores and blisters in the mouth. The neutropenia and its consequences can often be overcome by administering recombinant granulocyte-colony stimulating factor (G-CSF).

Treatment of CD40L deficiency is based on immunoglobulin replacement therapy, prophylaxis with trimethoprim-sulfamethoxazole to prevent *Pneumocystis jirovecii* infection, and protective measures to reduce the risk of *Cryptosporidium* infection (such as avoiding swimming in lakes or drinking water with a high concentration of *Cryptosporidium* cysts). In spite of this, many patients with CD40L deficiency die in late childhood or adulthood of infections, liver disease, or tumors (lymphomas and neuroectodermal tumors of the gut). The disease can be cured by hematopoietic cell transplantation, and this treatment should be considered when HLA-identical donors are available and when the first signs of severe complications become manifest.

Few cases of CD40 deficiency have been reported. Its clinical and immunological features, and its treatment, are very similar to CD40L deficiency, but the disease is inherited as an autosomal recessive trait.

Questions.

1 *Dennis's B cells expressed IgD as well as IgM on their surface. Why did he not have any difficulty in isotype switching from IgM to IgD?*

2 *Normal mice are resistant to Pneumocystis jirovecii. SCID mice, which have no T or B cells but have normal macrophages and monocytes, are susceptible to this microorganism. In normal mice, Pneumocystis jirovecii organisms are taken up and destroyed by macrophages. Macrophages express CD40. When SCID mice are reconstituted with normal T cells they acquire resistance to Pneumocystis infection. This can be abrogated by antibodies against the CD40 ligand. What do these experiments tell us about this infection in Dennis?*

3 *Why did Dennis make antibodies against blood group A and B antigens but not against tetanus toxoid, typhoid O and H, and streptolysin antigens? Would he have made any antibodies in response to his Streptococcus pyogenes infection?*

4 *Most IgM is circulating in the blood, and less than 30% of IgM molecules get into the extravascular fluid. On the other hand, more than 50% of IgG molecules are in the extravascular space. Furthermore we have 30–50 times more IgG than IgM in our body. Why are IgG antibodies more important in protection against pyogenic bacteria?*

5 *Newborns have difficulty in transcribing the gene for CD40L. Does this help to explain the susceptibility of newborns to pyogenic infections? Cyclosporin A, a drug widely used for immunosuppression in graft recipients, also inhibits transcription of the gene for CD40L. What does this imply for patients taking this drug?*

Doctor Notes

"Failure to class switch"

- Hyper IgM immunodeficiency is a hereditary deficiency of the CD40L on the X chromosome ⇨ males are more affected
- Results in absence of CD40L on patient T-cell *"T-helper most probably"*
- In contrary to **MHC II deficiency** where the problem is not in the gene but in the transcription factor!

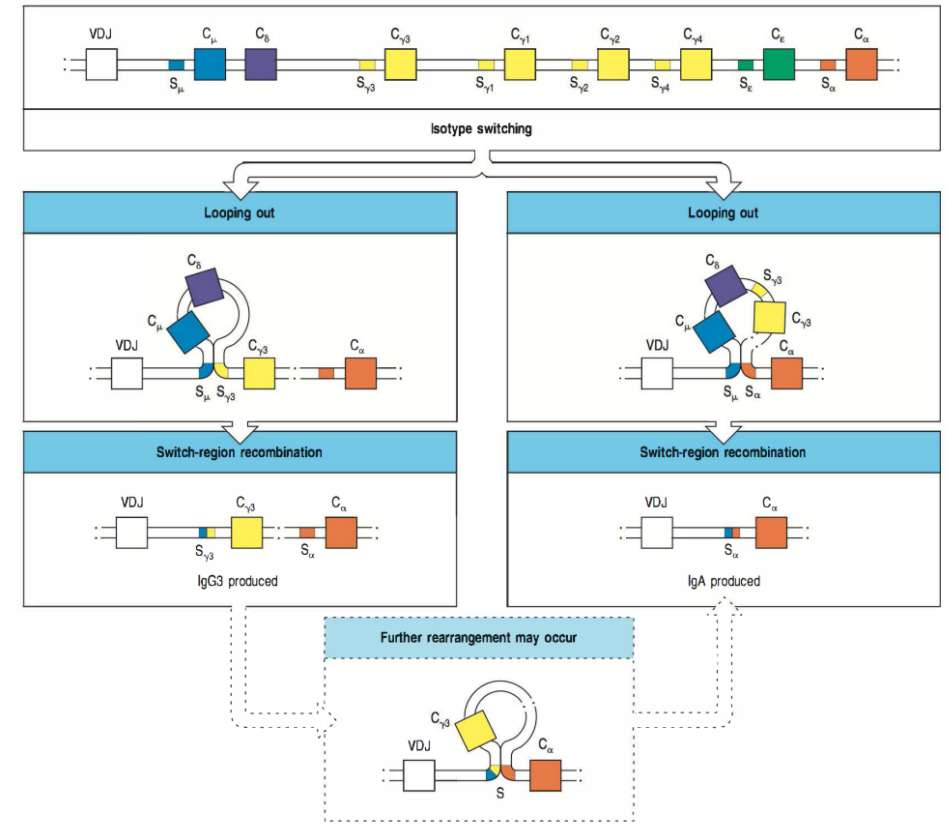
Effects both **Humoral** and **Cell Mediated** immunity because it effects both B-cells and T-cells activation

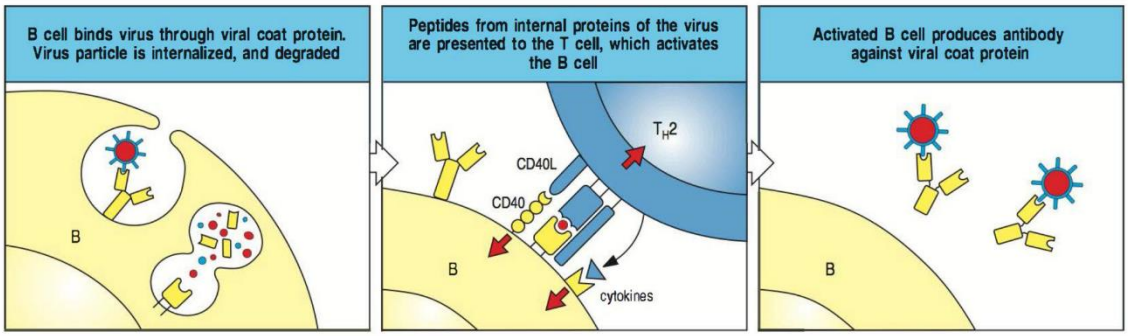
IgM is not good at opsonizing unlike IgG which considered a very good opsonizer ! *"IgG1"*

From this figure it's clear that IgM and IgD don't have to be looped to be produced so we don't need class switching to produce IgM and IgD , so no special enzymes are needed, only alternative splicing occurs "either M or D"

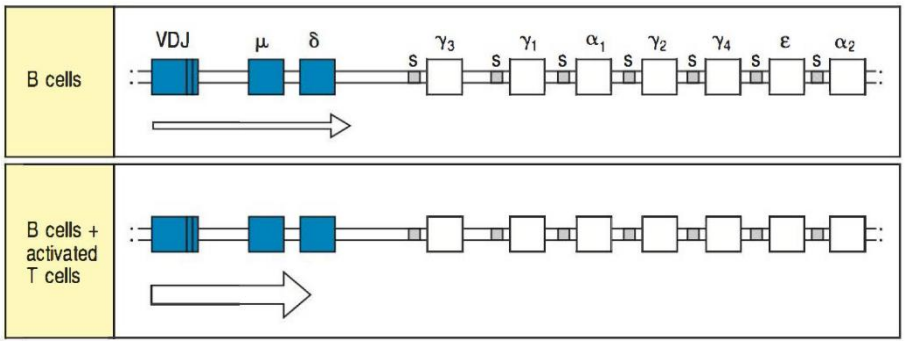
These enzymes that loops the DNA are responsible for class switching and they somehow get activated by CD40-CS40L binding

"IgM & IgD don't require activation by CD40L to be produced"





From this figure it's obvious that the B-cells secrete little amounts of IgM & IgD *"notice the narrow arrow"*



If the B cell is immature it will only produce surface antibodies, it will not secrete antibodies until it gets activated to a plasma cell "which is defective in CD40L deficiency "

Some information about Dennis Fawcett:

"Recurrent infections"

- Pneumocystis Carini "pneumocystis jirovecii": this organism is opportunistic, so whenever we see it means there is immunodeficiency "cell mediated immunodeficiency"
- β-hemolytic streptococcus normally found in nose and throat "very common for upper respiratory infections"
  - ASO titre is an antibody tests for this organism "anti-streptolysin O"
  - Why is it important to detect them?
    They could cause **Rheumatic fever** "cross-reaction with heart valves!" auto-immune disease

Anti-streptolysin O (ASO or ASLO) is the antibody made against streptolysin O, an immunogenic, oxygen-labile streptococcalhemolytic exotoxin produced by most strains of group A and many strains of groups C and G *Streptococcus* bacteria. The "O" in the name stands for *oxygen-labile*; the other related toxin being oxygen-stable streptolysin-S. The main function of streptolysin O is to cause hemolysis (the breaking open of red blood cells) — in particular, beta-hemolysis.

Increased levels of aso titre in the blood could cause damage to the heart and joints. In most cases, penicillin is used to treat patients with increased levels of aso titre.

-wikipedia

"The antibodies against them will cross react and attack the valves of the heart which is very serious and dangerous!"

Despite these infections WBC count is normal, this indicates a problem in activation of immune system

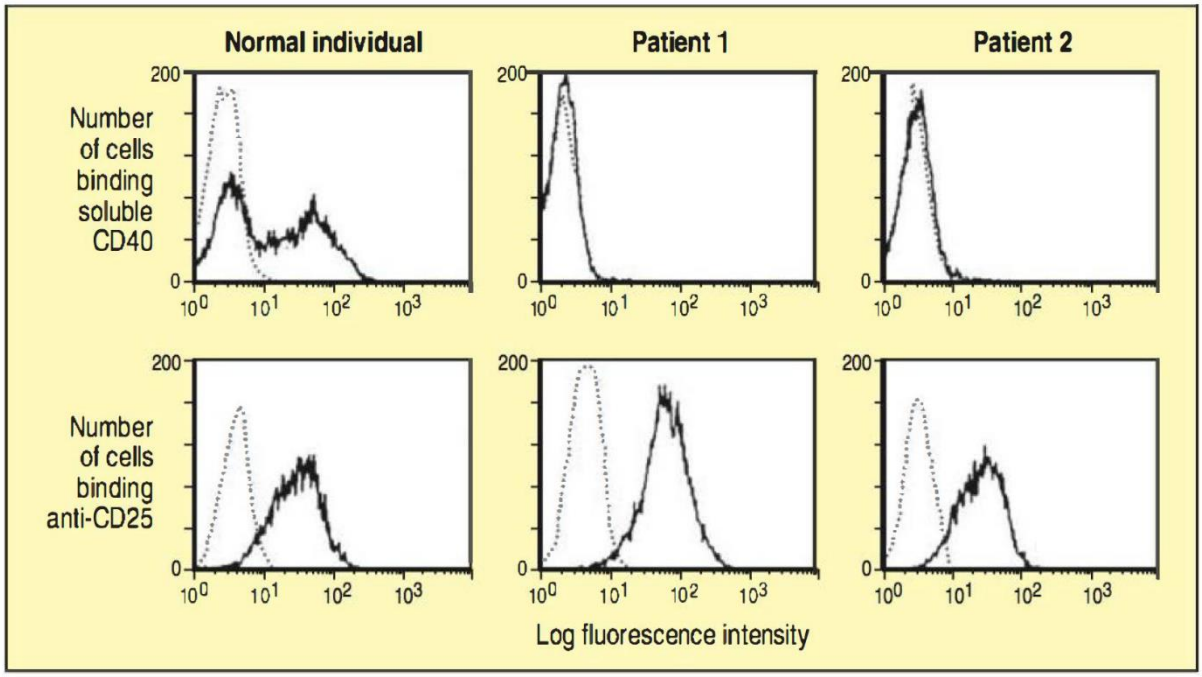
Ig levels:

> Remember:
>
> CD3 is a marker for T-cells and CD19 for B-cells

- High IgM level but low levels of other Immunoglobulins
- Dennis has type O blood group, IgM is the type of antibody which reacts against A and B *"so cross reaction can occur normally if wrong blood group is given"*
- Normal people with O type already have antibodies against A and B even without being exposed to them, how?

  There's a bacteria in the intestine which have similar antigens "sugars that mimic A and B" on it, so the immune system will recognize these antigens and produce antibodies against them.

FACS: fluorescence-activated cell sorting:



- Using fluorescent CD40
- Two patients and one normal individual
- We take T-cells from them, activate these cells <u>in vitro</u> to let them produce CD40L "if they can"
- Add labeled CD40 "fluorescent" to bind to the CD40L

- If T-cells have CD40L they will bind to labeled CD40 and produce higher fluorescence intensity "second peak of the normal person"
- If they're unable to produce CD40L, CD40 won't bind to them giving a result similar to the negative control "the dotted line"
- Normal persons have two peaks because **not all cells produce CD40L**.
- CD25 in the figure "second row" is a marker for activated T-cells to make sure that T-cells are activated ! Whether they have CD40L or not.

Negative Control: is a labeled peptide that is known to have no ability to bind to the cells, so it will give us a background negative signal

---

- Defects in Humoral immunity "antibodies" makes the individual prone to Pyogenic infections "Pus forming":
  - H. Influenzae, S. Pneumonia, S. Pyogenes, S. Aureus

(require opsonization to be phagocytosed)!! IgG is needed here "especially IgG1"

Low neutrophil count?

CD40L has a role in activating Macrophages and inducing them to secrete GM-CSF (Neutropenia)!

Macrophages activates t cell through two signals:

1) MhC

2) CD40 *"or B7"* (co-stimulation)

But this will also activate the macrophage itself to secrete the GM-CSF

- Defects in Cellular immunity leads to opportunistic infections
  - Ex: pneumocystis carinii which we talked about earlier

☆ Summary Questions:

If there is no class switch, how come patient's B cells express IgM and IgD?

There is no switch region between IgM and IgD

Why weren't patient's macrophages able to destroy P. carinii?

Activation of Macrophage to kill it require CD40-CD40L binding

Why do we have Ab against blood group but not tetanus or typhoid ags?

Blood ags are sugar groups= Can activate B cells in a T-cell independent manner. Tetanus and typhoid antigens are proteins= B cells require T cell help

Note: blood antigens are sugar moieties so the immune response to them is t-cell independent, because t- cells only recognize peptide antigens while b cells recognize both

Why is IgG crucial in opsonization and not IgM?

Fc receptors on phagocytes are directed against Fc portion of IgG and not IgM

How does Cyclosporin A work in graft recipients?

Inhibits transcription of CD40L gene. *"No co-stimulation and no activation of the immune system"*

Inhibits transcription of IL-2 gene *"IL2 is for proliferation of immune cells so it will inhibit the immune system in this way"*

=Increased susceptibility to pyogenic and opportunistic infections.

Used for transplant operations as immunosuppressant to inhibit rejection of the new organ

Mode of inheritance?

X-linked recessive

Similar case but autosomal recessive= CD40 deficiency

Other Questions:

How did we Rule Out SCID or Omenn Syndrome ?

B-cells were detectable

How did we Rule Out MCH II deficiency?

Neurtopenia and normal leukocytes count , also we can notice that IgM was high.

Why did he show reaction against A and B blood types, but Not against the DPT toxiod?

Since response against the blood types didn't require B cell activation so in that case it was independent activation however we need T-cell dependent activation for the DPT .

He had High IgM however he was still susceptible to infections ,why ?

Since IgM is a mainly for activation of complement system and some neutralization so at some point the bacteria will be able to overcome the complement system and causes infection, however the one responsible for opsonization and phagocytosis of pyogenic bacteria is IgG

Will newborn show symptoms early in life ?

No, because he will have IgG and IgA from his mother as passive immunity .

Why did the patient have high monocytes count ?

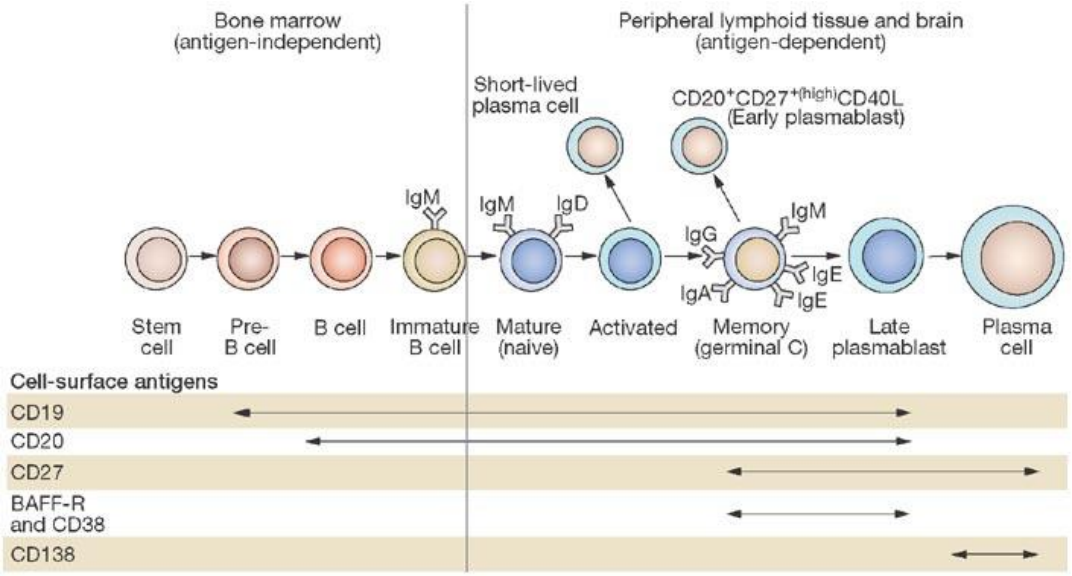
Since the body is trying to compensate and fight infections using the innate immune system .

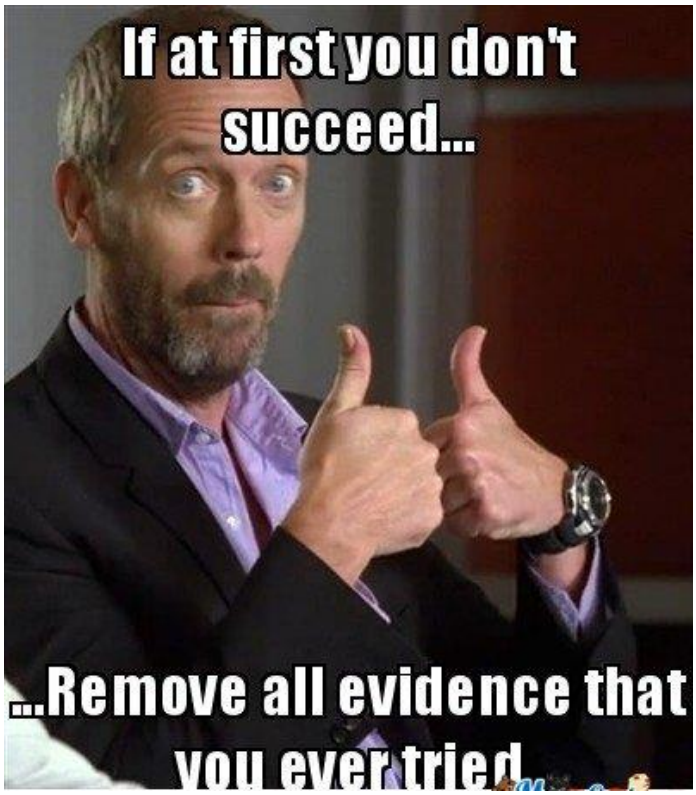
Remember: maturation vs. activation of B cells

Immature B cell in bone marrow VDJ recombination is finished here yet the only Immunoglobulin on the surface is IgM.

When these cells go out to the secondary lymph organs both IgM and IgD are present now on the surface and the B-cells here are called **Mature B-cells**

Activation of these cells occurs when they recognize an antigen and transform into antibody secreting **Plasma cell**





Le Fin.

Omar Saffar