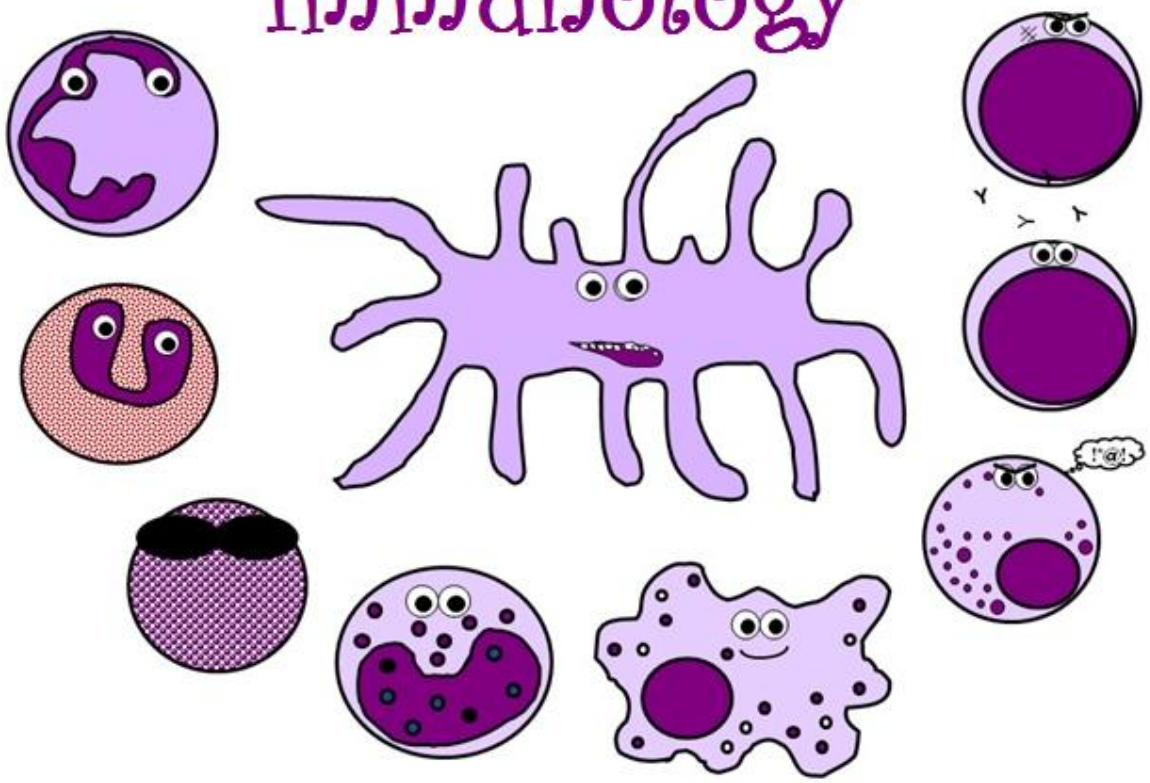
Immunology



Sheet Slides

Number: 7

Subject: MHC II Deficiency

Done by: Hashim Ahmad

Corrected by: correction team

Doctor: Issa Abi Dayya

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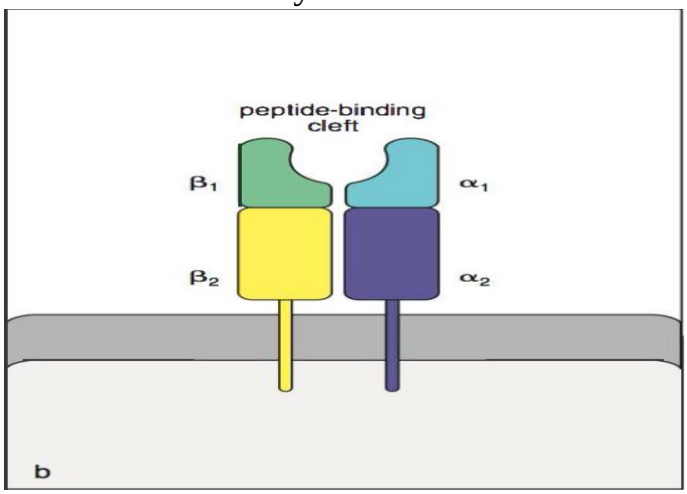
How to study this sheet?

- Read the introduction quickly (don't consume time memorizing it). Then, read the case twice and try to highlight the key things important in diagnosis. Then read the explanation of the disease at the end of the sheet. At the end, when you come back to the introduction and the case, they will be simple and common sense.

MHC II Deficiency

Introduction

- MHC II molecules are involved in presenting antigens to CD4 + T cells.
- The peptide antigens that they present are derived from extracellular pathogens and proteins taken up into intracellular vesicles, or from pathogens such as Mycobacterium that persist intracellularly inside vesicles.
- MHC class II molecules are expressed constitutively on antigen-presenting cells, including B lymphocytes, macrophages, and dendritic cells.
- In humans, together with the MHC class I molecules, they are known as the HLA antigens. They are also expressed on the epithelial cells of the thymus and their expression can be induced on other cells, principally by the cytokine interferon-gamma.
- T cells also express MHC class II molecules when they are activated.
- MHC class II molecules are heterodimers consisting of an alpha chain and a beta chain. The genes encoding both chains are located in the MHC on the short arm of chromosome 6 in humans. The principal MHC class II molecules are designated DP, DQ, and DR and, like the MHC class I mole-cules, they are highly polymorphic.



- Peptides bound to MHC class II molecules can be recognized only by the T-cell receptors of CD4 T cells and not by those of CD8 T cells.
- MHC class II molecules expressed in the thymus also have a vital role in the intrathymic maturation of CD4 T cells.
- Expression of the genes encoding the alpha and beta chains of MHC class II molecules must be strictly coordinated and it is under complex regulatory control by a series of transcription factors. The existence of these transcription factors and a means of identifying them were first suggested by the study of patients with MHC class II deficiency.

The case of Helen Burns: a 6-month-old child with a mild form of combined immunodeficiency.

- Helen Burns was **the second child** born to her parents. She thrived until 6 months of age when she developed **pneumonia in both lungs**, accompanied by a **severe cough and fever**. Blood and sputum cultures for bacteria were negative, but a **tracheal aspirate revealed the presence of abundant Pneumocystis jirovecii**. She was treated successfully with the anti-Pneumocystis drug pentamidine and seemed to recover fully.

- Pneumocystis jirovecii is an opportunistic pathogen, so this infection indicates an immunodeficiency status.
- As her pneumonia was caused by the opportunistic pathogen P. jirovecii, Helen was **suspected** to have **severe combined immunodeficiency**.
- If the child has no T cells or B cells, then it's severe combined immunodeficiency. If not, then it's something else.
- In order to know whether T cells are present or not, we have to induce their proliferation by a substance called "mitogen". The non-specific mitogen used to induce T-cell proliferation is called Phytohemagglutinin PHA.

→ Now, we want to induce T-cell proliferation with PHA and measure that by ^{3}H-thymidine incorporation assays to know whether it's SCID or something else.

A blood sample was taken and her peripheral blood mononuclear cells (i.e. lymphocytes) were stimulated with phytohemagglutinin {PHA) to test for T-cell function by ^{3}H-thymidine incorporation into DNA (To measure the mitotic activity of a cell, we look at DNA synthesis. One way to look at it is by using radioactive nucleosides and then measure the amount of radioactivity. More radioactivity means more cell division and mitotic activity).

The Result:

Normal T-cell proliferation, with the ability to respond to nonspecific mitogens like PHA and inability to respond to specific antigens like tetanus toxoid.

A normal T-cell proliferative response was obtained, with her T cells incorporating 114,050 counts min-1 of ^{3}H-thymidine {normal control 75,000 counts min-1). Helen had received routine immunizations with orally administered polio vaccine and DPT {diphtheria, pertussis, and tetanus) vaccine at 2 months old. However, in further tests her T cells failed to respond to tetanus toxoid in vitro, although they responded normally in the ^{3}H-thymidine incorporation assay when stimulated with allogeneic B cells {6730 counts min-1 incorporated, in contrast with 783 counts min-1 for unstimulated cells).

To completely rule out severe combined immunodeficiency, we have to prove that there are T-cells.

→ Helen's white blood cell count was elevated at 20,000 cells/ microliter {normal range 4000- 7000).

Leukemoid Reaction

The Differential:

Of these, 82% were neutrophils, 10% lymphocytes, 6% monocytes, and 2% eosinophils.

Neutophilia and **lymphopenia**

The calculated number of 2000 lymphocytes was low for her age {normal >3000 cell). Of her lymphocytes, 27% were B cells as determined by an antibody against CD20 {normal 10-12 %), and 47% reacted with antibody to the T-cell marker CD3.

2000 Cell → 27% are B cells and 47% are T-cells

(T-cells are present, so it's not SCID).

In particular, 34% of Helen's lymphocytes were positive for CD8, and 10% were positive for CD4. Thus, at 680 cells, her number of CD8 T cells was within the normal range, but the number of CD4 T cells (200 cell) was much lower than normal {her CD4 T-cell count would be expected to be twice her CD8 T-cell count).

The presence of substantial numbers of T cells, and thus a normal response to PHA, ruled out a diagnosis of severe combined immunodeficiency

Until now, we concluded that Helen's case is not SCID but we didn't know what it actually is.
Now, we will know. See how it was discovered!

Helen's pediatrician referred her to the Children's Hospital for consideration for a bone marrow transplant, despite the lack of a diagnosis (the pediatrician doesn't know what the disease is. He just knows that it's not SCID).

Remember:

MHC I → HLA-A,B and C
MHC II → HLA-D (DQ. DR and DP).

- Now, there are three possible donors, which are the parents and her healthy 4-year-old brother. However, when an attempt was made to HLA-type Helen, her parents, and her healthy 4-year-old brother by serology, **a DR type could not be obtained from Helen's white blood cells**. Her circulating B lymphocytes were transformed with the Epstein-Barr virus {EBV) to establish a B-cell line, which was then analyzed by flow cytometry. The EBV-transformed B lymphocytes **did not express HLA-DQ or HLA-DR molecules**. Hence, **a diagnosis of MHC class II deficiency was established. (Compare the levels of HLA-DQ and HLA-DR between normal individuals and Helen).**

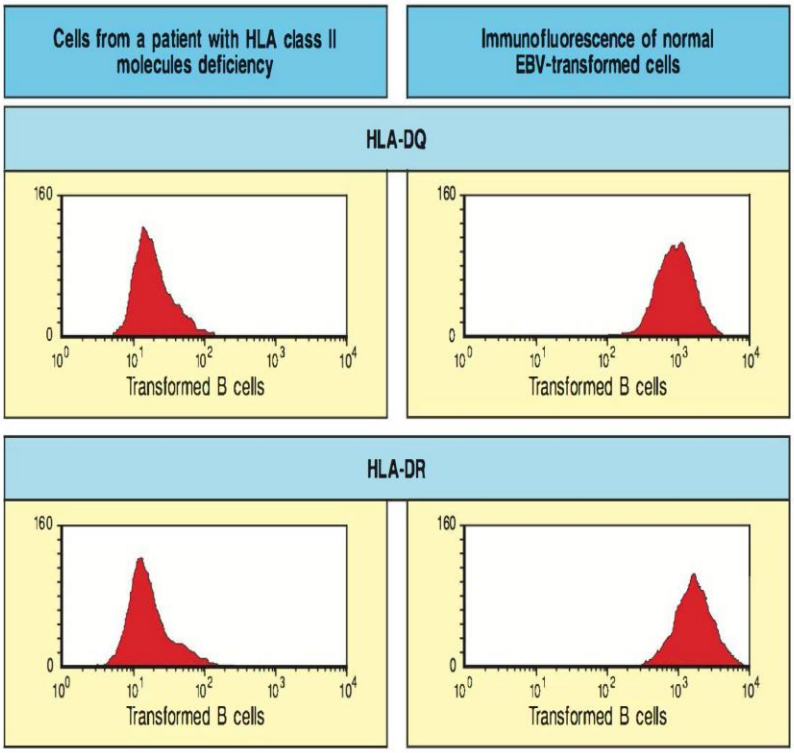


Fig. 8.4 Detection of MHC class II molecules by fluorescent antibody. Helen's transformed B-cell line was examined by using a fluorescent antibody against HLA-DQ and HLA-DR. Helen (left panels) expressed approximately 1% of the amount of MHC class II molecules compared with a transformed B-cell line from a normal control (right panels).

- Her brother was found to have the same HLA type as Helen, and therefore was chosen as a bone marrow donor. Helen was given 1 mg kg-1 of body weight of the cytotoxic drug busulfan every 6 hours for 4 days and then 50 mg kg-1 cyclophosphamide each day for 4 days to ablate her bone marrow. The brother's bone marrow was adminis-tered to Helen by transfusion without any in vitro manipulation. The graft was suc-cessful and immune function was restored.

MHC class II deficiency.

This is a summary of MHC II deficiency. If you have understood the case, they will be very easy for you. Please, if you are still not well-understanding the case, go back and read it again.

- MHC class II deficiency is inherited as an **autosomal recessive** trait.
- **<u>Health problems show up early in infancy</u>**.

- Affected babies present the physician with **a mild form of combined immunodeficiency** as they **have increased susceptibility to pyogenic and opportunistic infections.**
- They differ from infants with severe combined immunodeficiency in that **they have T cells, which can respond to nonspecific T-cell mitogens such as PHA and to allogeneic stimuli**.
- Unlike in some other types of immunodeficiency, progressive infection with the attenuated live vaccine strain BCG has not been observed in MHC class II-deficient patients after BCG vaccination against tuberculosis (most cases of MHC class II deficiency have been observed in North African migrants in Europe, where BCG vaccination is routine). This is because mycobacterial antigens derived from BCG can be presented on MHC class I molecules and infected cells can be destroyed by cytotoxic T cells. In contrast, and for reasons that are unclear so far, **patients with MHC class II deficiency are highly prone to severe viral infections**.

- Patients with MHC II deficiency also have **moderate to severe hypogammaglobulinemia.**

Treatment of MHC II Deficiency:

- Hematopoietic stem cell transplantation is the treatment of choice.
- Helen was cured after the bone marrow transplant from her HLA-identical donor.
- However, bone marrow transplantation is often not satisfactory even when transplanted from HLA-identical donor. What explains that?

  In MHC II deficiency, even after bone marrow transplant, there's still a deficiency of MHC II in the epithelial cells of the thymus, which are responsible for presentation of self-antigens, including MHC II to the developing T cells. So, if the developing T cells fail to recognize MHC II, they will die by apoptosis (Failure of Positive Selection).

Remember: Positive-selection is the selection of developing T-cells that are able to recognize MHC I or MHC II.
Negative-selection is the disposal of self-reactive lymphocytes that either bind self-peptides or MHC molecules with high affinity.

Genetics of MCH II Deficiency

- Whenever we want to know the gene responsible for a certain genetic disease, we start with linkage analysis (Sheet 35 Genetics :P).
- MHC II gene is present on the short arm of chromosome 6. However, linkage analysis showed that this condition is not linked to this locus!!
- Moreover, normally interferon-gamma induces the expression of MHC II on APCs, but here in this condition it doesn't.
  What does that mean?!

This means that the gene is intact, but there's a problem in the regulation of expression of MCH II genes.

Now, we know that the problem is in the regulation of expression rather than the genes themselves.
Then, we should search for the cause of the defect.

- While scientists were searching for the cause of the defect, they noticed something weird. When they fuse B-cell lines from two different patients, the two cells express MHC II. This proves that different patients have

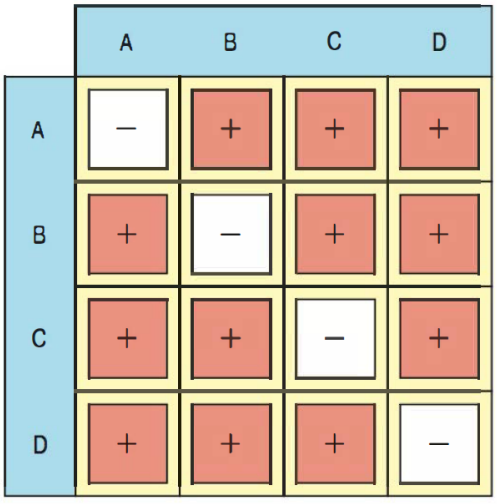


Fig. 8.5 Complementation groups of MHC class II deficiency. B-cell lines isolated from different patients were fused in all pairwise combinations to determine whether they could correct each other's defect. If two cell lines do not correct each other (–), they are in the same complementation group and have the same genetic defect. However, if the defect is corrected (+), the two cell lines belong to two different complementation groups and have two different defects. Four complementation groups, A, B, C, and D, were discovered by this technique.

different defects and that they corrected each other's defects after fusion.

- Look at the figure.

Four complementation groups were identified. This led to the identification of the defect.

→ The defect in MHC II deficiency results from defects in the transcription factors required to regulated their expression.

Questions:

1 Why did Helen lack CD4 T cells in her blood?

2 Why did Helen have a low level of immunoglobulins in her blood?

3 In SCID, lymphocytes fail to respond to mitogenic stimuli. Although Helen was first thought to have SCID, this diagnosis was eliminated by her normal response to PHA and an allogeneic stimulus. How do you explain these findings?

4 If a skin graft were to be placed on Helen's forearm do you think she would reject the graft?

5- How was SCID ruled out? Helen's T cells are low in numbers but normal. They can respond to non-specific mitogens such as PHA, or allogenic stimulus with Ag presented on foreign MHC.

6- Why no response to tetanus toxin? Cells were unable to present it on MHC II molecules to CD4 T cells.

7- Would Helen reject a skin graft? Yes. Her T cells are functional and able to recognize foreign MHC molecules on the grafted skin.

8- Would a SCID patient reject the skin graft? Most likely no.

Answer 1

The maturation of CD4 T cells in the thymus depends on the interaction of thymocytes with MHC class II molecules on thymic epithelial cells. When the MHC class II genes are deleted genetically in mice, the mice also exhibit a deficiency of CD4 T lymphocytes.

Answer 2

The polyclonal expansion of B lymphocytes and their maturation to immunoglobulin-secreting plasma cells requires helper cytokines, such as IL-4, from CD4 T cells. Helen's hypogammaglobulinemia is thus a consequence of her deficiency of CD4 T lymphocytes.

Answer 3

Helen's T cells, although decreased in number, are normal and are not affected by the defect. They are capable of normal responses to nonspecific mitogens and to an allogeneic stimulus in which the antigen is presented by the MHC molecules on the surface of the (nondefective) allogeneic cells and thus does not require to be processed and presented by the defective cells. However, the failure of her lymphocytes to respond to tetanus toxoid *in vitro* resulted from the fact that, in this situation, there were no cells that could present antigen on MHC class II molecules to the CD4 T cells.

Answer 4

Yes. Helen's T cells would be capable of recognizing the foreign MHC molecules on the grafted skin cells and would reject the graft.