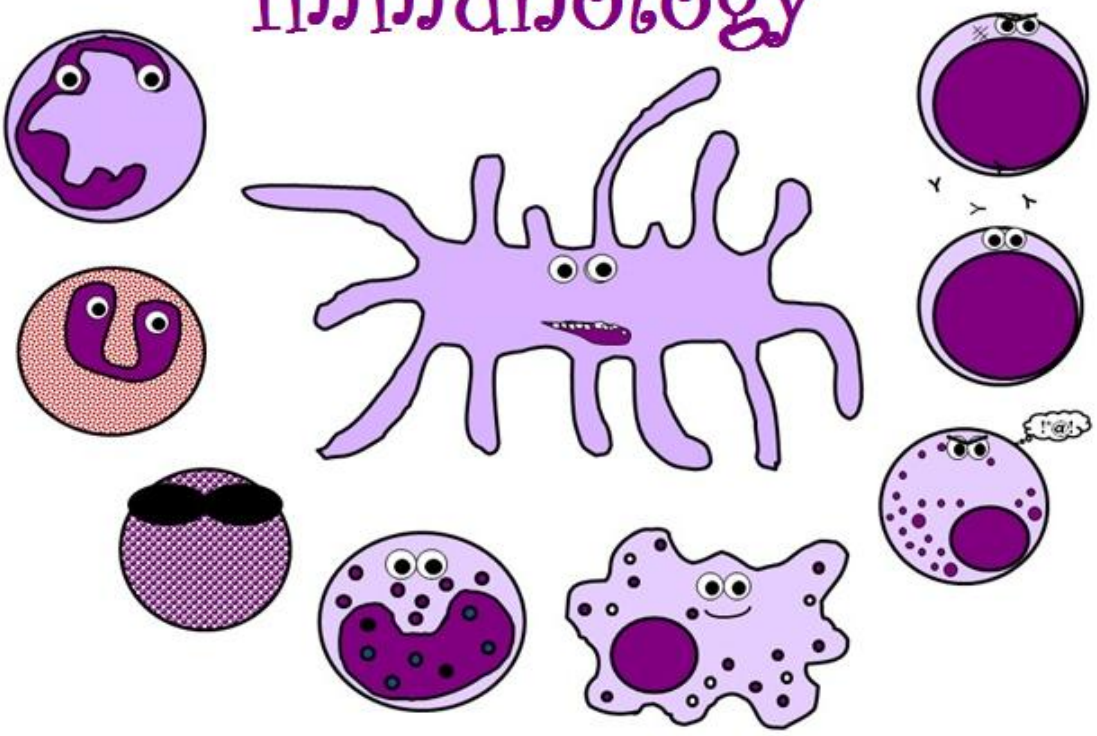
Immunology



Sheet Slides

Number: 21

Subject: SLE: a case study

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Please read the following book pages before proceeding to the lecture sheet:

A disease caused by immune complexes.

Immune complexes are produced whenever there is an antibody response to a soluble antigen. As the immune response progresses, larger immune complexes form that trigger the activation of complement. These activated complement components then bind the triggering immune complexes. Large complexes are efficiently cleared by binding to complement receptor 1 (CR1) on erythrocytes, which convey the immune complexes to the liver and spleen. There, they are removed from the red-cell surface through interaction with a variety of complement and Fc receptors on Kupffer cells and other phagocytes (Fig. 37.1). When antigen is released repeatedly, there may be a sustained formation of small immune complexes; these complexes tend to be trapped in the small blood vessels of the renal glomeruli and synovial tissue of the joints.

The most common immune-complex diseases are listed in Fig. 37.2. In subacute bacterial endocarditis, bacteria reside for a protracted period on the heart valves. This infection and subsequent inflammation damage the valve. At the same time, the antibody response to the prolonged presence of the bacteria is intense, and immune complexes of IgG antibodies and bacterial antigens are formed. These complexes become trapped in the renal glomeruli and cause glomerulonephritis. The immunoglobulins in the immune complexes provoke the formation of anti-IgG IgM antibodies known as rheumatoid factor (see Case 36). In a similar fashion, viral hepatitis can become a chronic infection that provokes a marked IgG antibody response, with the consequent formation of virus-containing immune complexes and rheumatoid factor. The immune complexes can be entrapped in the renal glomeruli as well as in small blood vessels of the skin, nerves, and other tissues, where they cause inflammation of the blood vessels (vasculitis). The antibodies in the virus-containing immune complexes have the property of precipitating in the cold (less than 37°C) and are therefore termed cryoglobulins (see Case 38).

This case was prepared by Raif Geha, MD, in collaboration with Erin Janssen, MD.

Topics bearing on this case:

- Clearance of immune complexes by complement
- Immune-complex disease
- Coombs' tests

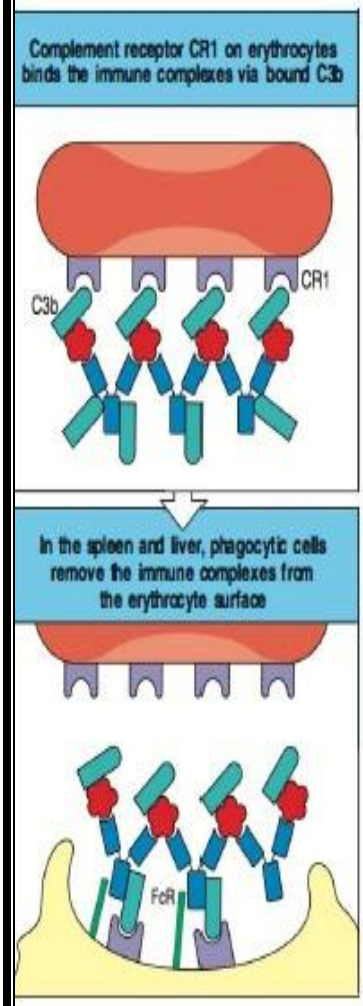

Fig. 37.1 Immune complexes are cleared from the circulation by binding to complement and Fc receptors. Immune complexes activate C3 in the serum, and bind activated complement components C3b, C4b, and C2a. C3b binds to complement receptors on erythrocytes, which transport the immune complexes to the spleen and liver, where complement receptors and Fc receptors on phagocytic cells bind to complement components and to the Fc portion of antibodies, and are thereby stimulated to engulf the complexes and degrade them.

The most prevalent immune-complex disease is systemic lupus erythematosus (SLE), which is characterized by the formation of antibodies against DNA. Every day, millions of nuclei are extruded from erythroblasts in the bone marrow as they mature into red blood cells (erythrocytes). This event, among others, provides a rich source of DNA in those individuals prone to making an immune response to DNA and developing SLE.

The case of Nicole Chawner: too much sun at the beach.

Nicole Chawner was a healthy 16-year-old until this summer. A few days after excessive exposure to the sun on the beach, Nicole developed a red rash on her cheeks. She saw her family doctor, who recognized that the butterfly rash on her cheeks and bridge of her nose was typical of systemic lupus erythematosus (SLE) (Fig. 37.3).

He referred Nicole to the Children's Hospital, where she was asked about any other problems she might have noticed. Nicole said that when she woke up in the morning her fingers and knees were stiff, although they got better as the day wore on. Nicole had also noticed some symmetric swelling in her fingers.

A blood sample was taken from Nicole to ascertain whether she had anti-nuclear antibodies (ANA). These were positive, at a titer of 1:1280. Because of this result, further tests were performed for antibodies characteristically found in SLE. An elevated level of antibodies against double-stranded DNA was also found. Her serum C3 level was 73 mg dl^{-1} (normal 100–200 mg dl^{-1}). Her platelet count was normal at 225,000 μl^{-1}, and her direct and indirect Coombs tests were negative, as was a test for anti-phospholipid antibodies. A urine sample was also found to be normal.

Nicole was advised to take an antimalarial agent, hydroxychloroquine sulfate (Plaquenil), and to avoid direct sunlight. She did well for a while but, after a month, the

morning stiffness in her fingers and knees worsened. She developed a fever of 39°C each evening accompanied by shaking chills. Enlarged lymph nodes were felt behind her ears and in the back of her neck. She also lost 4.6 kg over the course of the next 2 months.

When she returned to the hospital for a check-up, it was noted that her butterfly rash had disappeared. She had diffuse swelling of the proximal joints in her fingers and toes. Blood was drawn at this time, and the level of anti-DNA antibodies was found to have increased. The serum C3 level was 46 mg dl^{-1}. Nicole was advised to take 10 mg of prednisone twice a day, as well as 250 mg of the nonsteroidal anti-inflammatory drug naproxen twice a day. This quickly controlled her symptoms, and she remained well. At her next visit, her serum C3 level was 120 mg dl^{-1}.

Fig. 37.2 Three autoimmune diseases that result in damage by immune complexes.

Immune-complex disease		
Syndrome	**Autoantigen**	**Consequence**
Subacute bacterial endocarditis	Bacterial antigen	Glomerulonephritis
Mixed essential cryoglobulinemia (see Case 38)	Rheumatoid factor IgG complexes (with or without hepatitis C antigens)	Systemic vasculitis
Systemic lupus erythematosus	DNA, histones, ribosomes, snRNP, scRNP	Glomerulonephritis, vasculitis, arthritis

Systemic lupus erythematosus (SLE).

Systemic lupus erythematosus (SLE) is the most prevalent immune-complex disease in developed countries. For reasons that are not clear, it affects 10 times as many females as males. Patients with SLE usually have antibodies against multiple autoantigens. The most common autoantibody, which is found in the serum of 60% of all SLE patients, is against double-stranded DNA. Other commonly found antibodies are against small ribonucleoproteins. Autoantibodies against blood cells, such as platelets and red blood cells, as well as against the phospholipid complex that is formed by the activation of the proteins of the clotting system (antiphospholipid antibodies), are not infrequently seen. Most patients tend to have a range of these autoantibodies.

The immune complexes in SLE are small and tend to be trapped or formed inside tissues, primarily in the kidney and, to a lesser extent, in the synovial tissues of joints. For this reason, glomerulonephritis and arthritis are two of the most frequently encountered symptoms of SLE. These immune complexes fix complement efficiently, and tissue injury to the kidney or joints is mediated by activation of the complement system.

Cytokine signaling pathways have also been implicated in the pathogenesis of lupus. Type I interferons (IFN-α and IFN-β), which are important in suppressing viral replication, are secreted in response to the triggering of Toll-like receptors (TLRs). Immune complexes may trigger this response in SLE. Type I IFNs promote the activation of autoreactive T cells and augment class switching and antibody production in B cells. IRF5 is a transcription factor involved in interferon synthesis, and IRF5 haplotypes were one of the first genetic susceptibility factors identified in SLE. Further support for the link between IFNs and SLE is provided by the fact that a small percentage of patients on IFN-α treatment develop lupus. This drug-induced SLE occurs regardless of gender and tends to clear once the IFN-α is withdrawn.

The word 'lupus' is Latin for wolf, and this word is applied to a common symptom of SLE, the butterfly rash on the face. In the 19th century, the severe scarring rash on the face was named lupus because it was said to resemble the bite of a wolf. At that time, it was not possible to distinguish lupus erythematosus from lupus vulgaris, a scarring rash caused by tuberculosis. For unknown reasons, the rash is evoked by exposure to the sun (ultraviolet light). There is a seasonal variation to the onset of SLE, which is greatest in the Northern Hemisphere between March and September, when the greatest amount of ultraviolet light penetrates the atmosphere. Antimalarials such as hydroxychloroquine seem particularly helpful in the treatment of lupus skin disease.

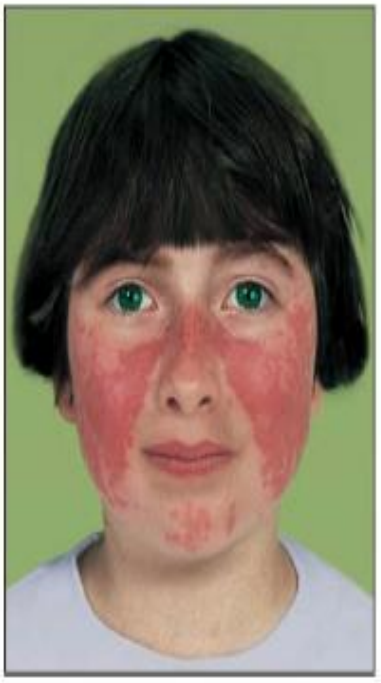

Fig. 37.3 The so-called butterfly rash typical of systemic lupus erythematosus. Photograph courtesy of M. Walport.

Questions.

1 Why do you think Nicole's serum C3 was measured, both on her first visit to the hospital and after therapy?

2 What are the direct and indirect Coombs tests, and what did they tell us in this case?

3 Why was Nicole told to avoid direct exposure to sunlight?

4 Repeated analysis of Nicole's urine was negative. What does this mean?

5 Nicole had a serum IgG level of 2020 mg dl^{-1}. This substantially elevated level of IgG is commonly found in patients with SLE. How could you explain this? And what would you expect to find if we took a biopsy of Nicole's swollen lymph nodes?

6 The antigen in the immune complexes formed in SLE is often a complex antigen, such as part of a nucleosome or a ribonucleoprotein particle, which contains several different molecules. Patients often produce autoantibodies against each of these different components. What is the reason for the production of this variety of autoantibodies, and what type of failure in tolerance could be responsible for autoantibody production?

Case 37

Answer 1

The serum levels of complement proteins C3 and *C4* are lowered in SLE by the large number of immune complexes binding C3 and *C4*, triggering their cleavage. The depletion of these proteins is therefore proportional to the severity of the disease. Successful immunosuppressive therapy is reflected in an increase in the serum levels of C3 and *C4*. Measurement of either C3 or *C4* is sufficient; it is not necessary to measure both, and C3 is most usually measured.

Answer 2

The objective of these tests was to establish whether Nicole had autoimmune hemolytic anemia, which occurs in SLE when there are antibodies against erythrocytes. Nicole did not have hemolytic anemia (see Case 41).

Answer 3

Because ultraviolet light provokes the onset of SLE and causes relapses.

Answer 4

She had not developed glomerulonephritis. If she had, her urine would have contained protein and red blood cells.

Answer 5

As a result of the constant stimulation of their B cells by autoantigens, patients with SLE have a greatly expanded B-cell population and consequently an increased number of plasma cells secreting immunoglobulin. A lymph node biopsy from Nicole would have exhibited follicular hyperplasia in the cortex and increased numbers of plasma cells in the medulla.

Answer 6

In the first place, a large multimolecular complex such as a nucleosome carries many separate epitopes, each of which can stimulate antibody production by a B cell specific for that epitope. Any of these antibodies can bind the nucleosome particle to form an immune complex. Such potentially autoreactive B cells probably exist normally in the circulation but, provided that T-cell tolerance is intact, they are never activated because this requires T cells to be reactive against the same autoantigen. SLE is probably caused by a failure of T-cell tolerance. T cells for each of the components of the complex antigen will not be needed to induce antibodies against its individual components. As Fig. A37.6 shows, a T cell that is specific for one protein component of a nucleosome could activate B cells specific for both protein and DNA components.

This sheet has been written based on the recording of section 1:

Systemic Lupus Erythematosus (SLE):

It is a type III immune response, meaning it is associated with immune complexes. The main problem here is that, these immune complexes reach small blood vessels, and also the synovial fluid of different joints (reason why it is considered systemic; many organs are affected), and are trapped there causing damage.

Besides SLE, there are many other examples on immune-complex diseases, of which are:

- **Sub-acute bacterial endocarditis**: a case in which bacteria is present on the heart valves for a long period of time, producing antigens that mimic auto-antigens, resulting in auto-immune diseases associated with immune-complexes that usually cause glomerulonephritis.

- **Cryoglobulinemia**: cryoglobulins are auto-antibodies precipitating under cold temperature, which explains the manifestation of certain immune diseases in cold weather, like Reynaud's phenomenon for example.
    - Certain viral infections, like Hepatitis C, can cause the body to produce cryoglobulins. (Cryo- means "cold")

Back to SLE:

In SLE we are talking about a complex antigen that contains DNA, histones, ribonucleoproteins etc… Antibodies can be formed against any of these constituents.

Normally, the human body gets rid of these immune complexes through the binding of these to certain complement receptors found on RBCs, those RBCs then travel to the secondary lymphoid organ where phagocytes recognize the FC portion of the antibodies within the immune complex-> phagocytosis and clearance.

If clearance fails and those complexes accumulate for whatever reason it is (increased number of the antigen possibly), they deposit and cause damage.

One of the very popular tests performed nowadays is the Food Intolerance test . It is found that there are two types of immune reactions against food: Type 1 which is food allergy, and food intolerance. What characterizes the latter is the following:

1) Not immediate, follows 2-3 days after the intake
2) IgG mediated, where those antibody-antigen complexes can deposit and cause a very broad spectrum of symptoms; arthralgia, headaches, flatus etc… It can also be the cause of an inability to lose weight, in which the intolerance is not necessarily against fatty food.

Establishing a diagnosis of SLE is not easy; it affects multiple body organs, and at the same time the symptoms can be generic. For this reason, they have created 11 criteria for classifying a possible SLE diagnosis. At least 4 of these criteria must be observed in the patient to consider it an SLE case, and as we said you will notice that these are very generic.

Of course, females are more commonly affected than men (a ratio of 9:1), and the earliest symptoms to appear are mostly skin-related:

- Malar rash (from Latin mala 'jaw or cheekbone'): butterfly rash (indicative of lupus erythematosus), but the rash won't necessarily manifest as a butterfly, it can be discoid in shape as well.

- Photosensitivity: as we have seen in the case of Nicole Chawner. For unknown reasons, exposure to UV light under the sun triggers symptoms.

- Oral ulcers.

Joint-related symptoms such as non-erosive arthritis, which means that it is not a case where one joint is undergoing aging related erosion, it is related to a systemic issue when at least two peripheral joints are involved.

Also within the criteria are pleuritis, pericarditis and renal disorders. Renal disorders are one of the most problematic consequences of SLE when left untreated, therefore, with Lupus patients urine tests must be continuously checked for; we need to make sure there is not persistent proteinuria, and we also check for the presence of **urine casts**.

If there is a problem in the reabsorption of proteins by nephrons, tube-like structures are excreted in the urine-- **urine casts**. Those can be either cellular (RBCs, WBCs) or acellular (proteins).

Neurologic disorders such as seizures and psychosis, and to make sure that these symtomps are not drug-induced or caused by metabolic diseases, a history must be taken.

Hematologic disorders: mostly anemia (notice the performing of Coomb's test in the case of Chawner), leukopenia and thrombocytopenia.

And finally, immunologic disorders; test for ANA (anti-nuclear antibody), antibodies against double-stranded DNA, and antibodies against Sm proteins (firstly discovered in the case of Stephanie Sm-ith, an SLE patient). According to the results of these tests, the diagnosis is established.

❖ ANA tests:

In brief, there are cells called Hep 2 cells; epithelial cells previously isolated from a laryngeal cancer patient, and transported onto laboratory slides. Then, the serum of the SLE patient is firstly diluted (if not diluted the result will definitely be positive), and then added on top of these slide. What we observe here are different titers, serial dilutions to quantify the amount of ANA in the serum of the patient.

For example, if the first dilution ratio is 1/80 and no signal is observed, then the results are negative. If there is any signal, we go further on diluting the serum to 1/160-> 1/320-> 1/640, and so on until no signal is omitted from the cells.

- ** the signal is the result of the interaction between the auto-antibodies (from patients serum) and cellular components (of the epithelial.)

In the lab report, the result will be represented as 'less than 1/80' in case it was negative.

If for example the signal was terminated at a titer of 1/1280, the titer preceding it is considered, 1/640 in this case. The higher the titer, the higher the concentration of the auto-antibody in the serum.

In this test, the cells not only florescent but also show patterns, some patterns are indicative of cytoplasmic signals. There are also **homogenous** patterns (the cell appears as if it has been sprayed), some are called speckled patterns (dotted); can be further classified, and others are called nuclear patterns.

- Patterns can help us to recognize the nature of the disease, for example in SLE it is either the homogenous pattern in which the double-stranded DNA and histones are most likely involved in the reaction, Or the speckled pattern where the antigen is the Sm protein.

Still, the ANA test is a screening test to make sure this is a case of an auto-immune disease, when the results are positive, they ask for another test that is more specific.

Notes:

- Sometimes elderly (over 60) can have elevated levels of ANA, without any underlying pathology.
- Generally, when the titer is above 1/320 we start suspecting the presence of an issue.

❖ Case of Nicole Chawner:

A 16 year old female patient, meaning that SLE can manifest at young ages. As a typical autoimmune disease, it affects females commonly, no specific reason explains this ratio but one of the theories suggests that female sex hormones can modify the immune system in way that makes more alert and therefore more prone to autoimmunity.

The butterfly rash upon sun exposure; as we said SLE patients are photosensitive. Patients must be warned about this trigger, as Lupus Erythematosus manifests as flares, it is very important to avoid triggering factors.

They performed an anti-double-stranded DNA test which was positive, notice that almost 60% of SLE patients have positive results for this auto-antibody. So, 40% of patients with Lupus are negative for the test, and that is why we have 11 criteria.

C3 and C4 levels are low due to overconsumption, and SLE is one of -if not the only- the diseases that are diagnosed with low levels of **both C3 and C4.** What is also important about these, is that their levels can be used for prognosis, meanwhile the ANA titer can't be used for this. There is no point for you as a doctor to ask your patient for second ANA tests.
So when the levels of C3 and C4 are elevated back it is an indication of improvement.

As for the hematological tests:
Platelet count was normal, and both **direct** and **indirect** Coomb's tests were negative.

- Direct: indicates the current presence of auto-antibodies attached to RBCs
- Indirect: Indicates the presence of a circulating immunoglobulin that can possibly attack RBCs and cause hemolytic anemia, example: Rh- compatibility between a pregnant mother and her baby:
    - When the mother is pregnant with Rh- positive baby, if their blood gets mixed and her body forms antibodies against Rh factor, during her second pregnancy and if the fetus is Rh-positive, she can pass those antibodies to her baby and cause them hemolytic anemia.

In such case, indirect coomb's test can be helpful.

A decent number of SLE patients they develop antibodies against phospholipid components, the presence of these antibodies makes males more prone to thrombosis, heart attacks, and strokes. As for females, besides thrombosis, heart attacks and strokes, it makes her more prone to recurrent abortions (they must be put on anti-coagulants immediately at the beginning of pregnancy).

Anti-malarial drugs are used for the treatment of SLE, how do they work exactly it is unknown, they just improve mostly the skin symptoms and the rheumatologic.

In case of Nicole Chawren they had to switch the treatment to steroid and NSAIDS as her conditions worsened.

In daily practice, do not expect the condition to be very clear and easily recognized, it can be very subtle so you have to be precise and attentive.

- ANA, anti-double stranded DNA, CBC, C3&C4, anti-phospholipid, inflammatory indices(ESR,CRP), urine analysis, are the tests you need to think of when you suspect a case of SLE.

- Why is it called Systemic Lupus Erythematosus?
  Systemic: affecting multiple organs.
  Lupus: means wolf, for the similarity between the rash on the face of SLE patients and the mark a wolf's bite would leave on a person's face.
  Erythematosus: red rash.

A very important note:

SLE can be drug-induced. IF-α treatment is one of the most common to induce Lupus, used in viral infections. Once the medication is discontinued, symptoms are relieved.

Also, the production of IF-α can be stimulated by IRF-5 (interferon regulatory factor) which is a transcription factor.

Different toll-like receptors present on the surface of the cell are linked to signaling pathways that lead eventually to the activation of IRF-5, which by turn induces the transcription of IF-α. IF-α is known to further activate the immune system to produce auto-antibodies that can make an SLE condition worse.

This explains the effect of IF-α treatment on SLE.

Some people have certain IRF-5 haplotypes that make them genetically predisposed to SLE.

Finally, the antigen of SLE is a complex antigen with many components, an antibody recognizing one of these components only, is enough to cause the production of antibodies against each of these different components, how is that?

- Once the antibody binds that component, the entire complex is taken into the B cell, then it is chopped into several pieces and those pieces are presented on the surface of the cell. So whether the T cell had specificity for the histones, the ribonucleoproteins or whatever, it will be able to provide the co-stimulation for that B cell to produce the antibodies.

In other words, a T cell that is specific for a histone for example, will be able to activate a B cell that recognizes the DNA molecule and thus produce auto antibodies.

Questions from the doctor's slides:

- What was C3 low, what is significance of multiple measurement?

C3 and C4 are low due to their binding and cleavage to immune complexes. The rise of their level is a sign of successful immunosuppressive therapy.

- Nicole's Urine test was normal, significance?

No glomerulonephritis, otherwise, proteinuria and RBCs in urine.

- Why elevated serum IgG?

Constant stimulation of their B cells by auto antigens.