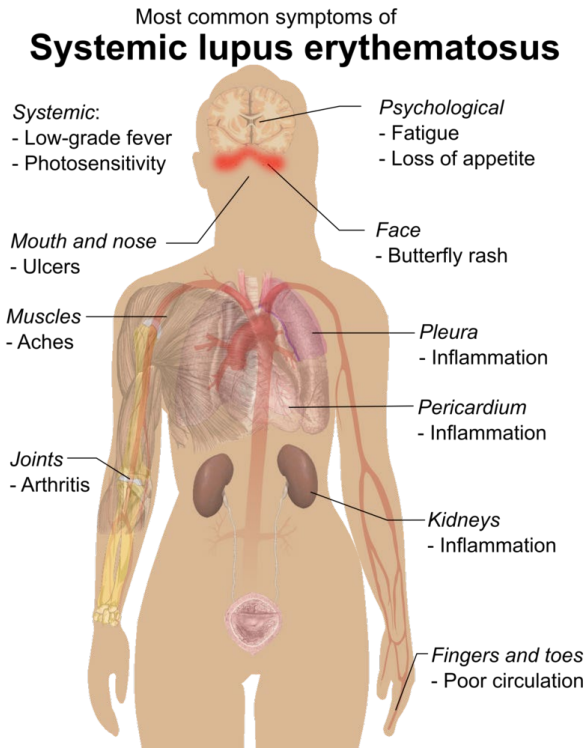
STANDARD DEVIATIONS: Parasites in the Lab

Greetings,

The regulation of immunity has garnered some attention with COVID-19. Cytokine storm and hyperactive response to infection are the suspected culprits in severe disorder that requires invasive therapies, like mechanical ventilation. The danger of hyperimmune activity in COVID-19 is bound to the virus and our effort to battle infection. *Auto-immunity* is another beast. Sometimes we attack our own body.

Systemic Lupus Erythematosus (SLE) is an autoimmune disease in which the body's immune system mistakenly attacks healthy tissue in different parts of the body. The Lupus Foundation of America estimates that 1.5 million Americans (> five million, worldwide) have a form of lupus. While joint pain and skin rash are the classic and predominant complaints, half the cases involve major organs (heart, lungs, kidney, and brain); and the psychological burden of fatigue can be debilitating.



The disparity in presentation is interesting. **Ninety percent are women** and studies (not much) indicate African American lupus patients are more likely to have organ system involvement, more active disease, and lower levels of social support compared with White lupus patients (American minorities are twice as likely to present with SLE).

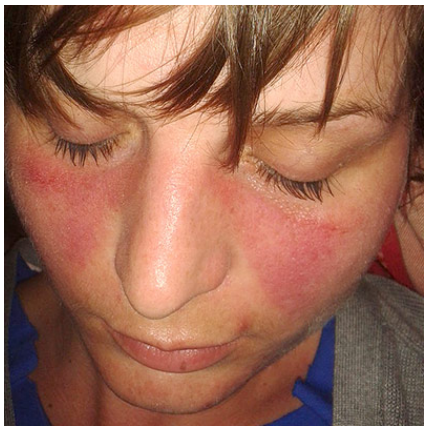
So, what's going on?

SLE is characterized by production of pathogenic autoantibodies directed against nucleic acids and their binding proteins. 97% are positive for antinuclear antibodies (ANA), but ANA isn't specific. The algorithm is to drill down in ANA (+) patients for more specific antibodies. The typical ANA panel includes Anti-double-stranded DNA antibody (anti-dsDNA), Anti-Smith antibody (anti-Sm), Anti-U1RNP antibody, and Anti-Ro/SSA and Anti-La/SSB. These tests enable the physician to tease out a diagnosis; here's how:

- **Anti-dsDNA Antibody.** Less than 1% of healthy individuals have this antibody but 70% of SLE patients will be positive. Anti-dsDNA antibodies often suggests more serious lupus, such as lupus nephritis (kidney lupus).
- **Anti-Smith Antibody.** This antibody is almost exclusively found in SLE and not other rheumatic disease, but only 20% of people with disease.
- **Anti-U1RNP Antibody.** Incidence of anti-U1RNP antibodies in people with lupus is around 25%, while less than 1% of healthy individuals possess it. Trouble is, it is not SLE specific and is found in other conditions.
- **Anti-Ro/SSA and Anti-La/SSB Antibodies.** These are antibodies found mostly in people with systemic lupus (30-40%) and primary Sjogren's syndrome and are often seen in ANA negative cases. These antibodies are not highly specific for systemic lupus; they are associated with other conditions (including sun sensitivity), and 15% of healthy folk have titers. Babies of SLE mothers with anti-Ro and anti-La antibodies are at an increased risk of neonatal lupus, a condition that produces a temporary rash and can lead to congenital heart block.

What about genetics?

There are several implicated genes under investigation but the multiple components make study difficult. Only 20% will have a parent or sibling affected, and only 5% of children born to positive mothers will develop disease. Why women (and minorities) are so disproportionately affected is still a mystery.



{Characteristic Malar (butterfly) rash in SLE.}

So, nailing down a diagnosis of SLE is not a simple process. In fact, because its symptoms mimic many other illnesses, lupus symptoms can be unclear, can come and go, and can change, it takes **nearly six years for people with lupus to be diagnosed**.

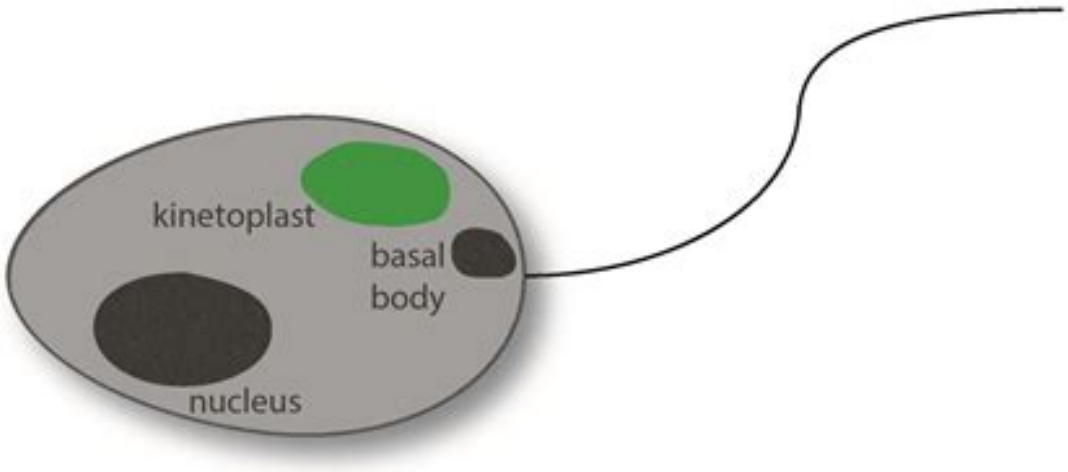
In lab time, that's an eternity.

But, the truth is that I've wasted a bunch of time just getting to the cool part of the newsletter. Our diagnostic tools for SLE rely heavily on the ANA panel, *and ds-DNA, in particular*. A positive ANA titer with presence of ds-DNA antibody is pretty indicative of SLE. And, as far as lab tests go, ds-DNA is unique. The rest of the post is about this neat little assay.

As implied, Anti ds-DNA targets double stranded DNA. The binding disrupts cellular progress and leads to cell death (apoptosis). The cell death triggers intense B-cell proliferation, interleukins and pathological T-B cell collaboration resulting in inflammation and tissue injury.

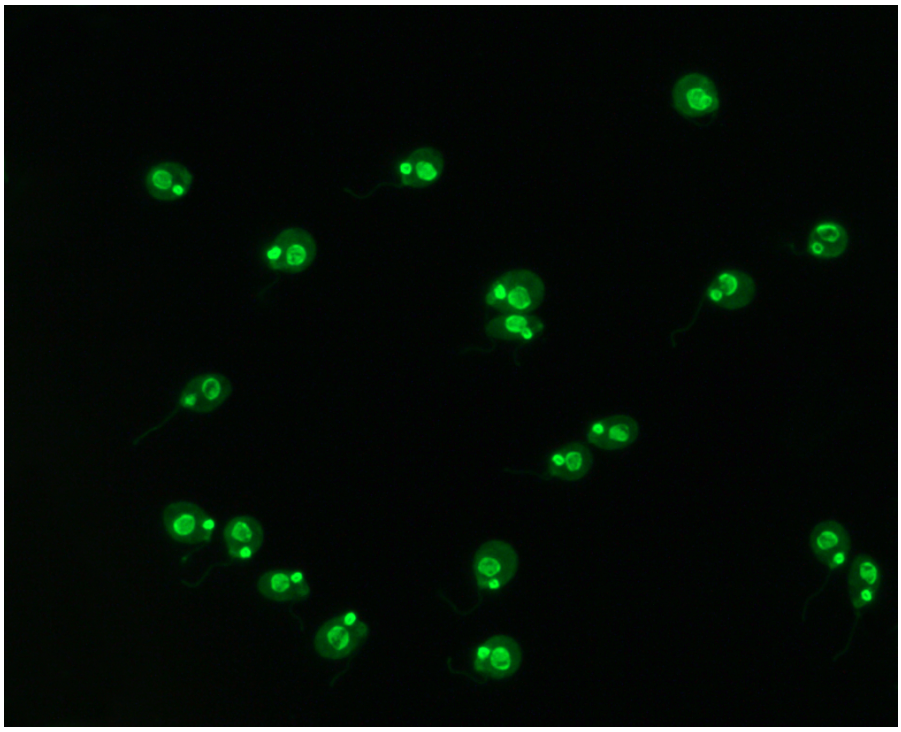
We test Anti ds-DNA with an indirect fluorescence assay, looking for a fluorescing complex (Ab bound to DNA). The fascinating part is the substrate we use as a reagent. The assay uses a flagellated parasite of houseflies, *Crithidia luciliae,* a trypanosome.

C. luciliae is a eukaryotic single-cell protozoan. The motor for the flagellum is called a kinetoplast, a network of interlocking circular DNA in a huge mitochondrion. Human sera containing Anti ds-DNA binds to the kinetoplast DNA. Then we tag that with FITC.



{Flagellated trypanosome model}

When the FITC-tagged complex is formed the resulting fluorescence is characteristic and looks like this under the microscope:



{The *C. luciliae* nuclear DNA and kinetoplast light up.}

SLE is a strange disease. It affects women and minorities but wavers in presentation, making it difficult to diagnose. SLE is a rheumatic auto-immune disorder presenting with rash, joint pain and fatigue and leads to complication in major organs. It's a burden to millions (mostly women) and there is no cure. The nature of its presentation delays diagnosis but we have a unique serology available to labs that targets the mitochondrial DNA of a housefly parasite.

We spend a lot of time diagnosing disease. We spend a lot of time developing diagnostic assays. We spend a lot of time and effort avoiding, treating and eradicating parasites. Sometimes we turn right around and use those parasites to do good laboratory science!

Have a great week and be safe,

Bryan