Modulation of Innate Immunity by Pathogens
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Lecture Notes courtesy of Mary Brunson

Dr. Emily Rosowski – “Modulation of Innate Immunity by Pathogens” (Tuesday, July 16, 2013) (Laboratory of Jeroen Saeij)

1. Infectious disease
   i. leading worldwide killer (15.3% of world deaths)
   ii. interplay between pathogen and host
   iii. Why do different hosts have different responses to infection? (HIV, e.g.)
      a. Pathogen genetic differences (better at replication, infection)
      b. Host genetic differences (different T-cell responses)
      c. Environmental differences (diet, etc)
   iv. Causative agents are mostly intracellular pathogens
      a. Invasion \(\rightarrow\) replication \(\rightarrow\) egress (leave the cell)

2. Toxoplasma gondii (single cell eukaryotic)
   i. Good model (can infect any warm blooded animal)
   ii. Easy to work with in lab (genetic/biochemical methods, easy to grow in tissue culture, easy to infect mice in vivo
   iii. Relevant to human disease (related to malaria, 30% of humans worldwide are infected)
   iv. Disease manifestations:
      a. Immune-competent (life-long chronic infection with retinal scarring, behavioral effects)
      b. Immune-compromised (flu-like symptoms, encephalitis, liver and heart problems)
      c. Congenital transmission (birth defects)

3. Strategies to survive and propagate different hosts
   i. Yersina pestis (plague blocks feeding of rat parasite to spread to different rats)
   ii. Trypanosoma brucei (different strains live in different hosts, but only make one host sick)

4. Transmission and sexual cycle of Toxoplasma
   i. Infect cell, replicate in a vacuole, then vacuole bursts, releasing parasites to infect other cells (tachyzoites)
   ii. Can infect any cells
   iii. Tachyzoites (fast-growing) convert to tissue cysts (slow-growing) when under stress (immune response, low pH, low CO2)
   iv. If cat eats tissue cysts (mostly in brain and muscles), the parasite starts meiosis to produce oocysts (millions)
   v. Other animals can be infected orally through tissue cysts (infected animal) or oocysts (feline feces)
   vi. Fast-growing stage parasites are not infectious
   vii. Balances its own survival with host survival
a. Complete immune invasion → High levels of tachyzoites → host dies
b. Strong immune response → parasite dies → host survives
c. Conversion to cysts → host survives to transmit the parasite

5. How does it modulate host processes?
   i. Secrete proteins into host cell
   ii. Micronemes create a pore into host cell, allowing dense granules and micronemes to enter cell
   iii. Moving junctions allows parasite to move into cell into a vacuole created entirely from host cell membrane (parasitophorous vacuole membrane)
   iv. Dense granule proteins can be exported from vacuoles and go to the host nucleus
   v. Three clonal lineages:
      a. Type 1 → LD100 = 1 parasite
      b. Type 2 → LD50 = 250 parasites
      c. Type 3 → LD50 = 100,000 parasites
      d. Differences in virulence due to how they modulate host cell gene expression
   vi. Use microarray analysis to test host gene expression
      a. mRNA has poly-A tail, so use labeled cRNA with poly-U tail to attach
      b. Use different DNA sequences (genes) on the chip to match up with cRNA – test 14,500 genes
      c. Type II strain specifically induces an expression of a subset of host genes
         a. Are these type II-induced genes controlled by a common transcription factor or pathway
         b. Nuclear factor-kappaB (nfb) is more common in these promoters
         c. Immune response is triggered more so in the type II-infected cells (in a culture dish)
         d. In Type II infection, NF-kB is located primarily in the nucleus, not true in Type III
      d. What Type II gene is responsible for phenotype?
         a. Type II and Type III (haploid) combine to form diploid oocyst (and crossover)
         b. Look at all F1 progeny and see which Type II genes (of the following candidates) are necessary for behavior:
            a. Needs to secrete proteins
            b. Polymorphic between I/III and II strains
            c. Expressed well in tachyzoites
            d. Polymorphic gene GRA15 is responsible (Add to type I and type III and it works, KO from type II and it doesn’t)
            e. 1,756 known host genes are regulated by GRA15
   vii. Toxoplasma growth is largely controlled by the Immunity-Related GTPases (mice die if they don’t have these and are infected)
      a. GRA15 induces IL-12 production, which activates a pathway that creates IRGS that fight off the toxoplasma
      b. GRA15 inhibits parasite growth
c. GRA15 activates a strong immune response, which acts on the right side of the balance (helping the mouse)

6. What factors are relevant in different hosts?
   i. Humans don’t have IRG proteins
   ii. Humans with mutations in IFN-gamma receptor don’t have increased susceptibility to *Toxoplasma*
   iii. Rats are resistant to the most virulent *Toxoplasma* strains in mice
   iv. New strains in South America can cause disease even in immune-competent humans