Published in final edited form as:

J Theor Biol. 2013 September 7; 332: 267–290. doi:10.1016/j.jtbi.2013.03.024.

Towards multiscale modeling of influenza infection

Lisa N. Murillo^a, Michael S. Murillo^b, and Alan S. Perelson^{a,*}

^aTheoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

^bComputational Physics and Methods, Los Alamos National Laboratory, Los Alamos, NM 87545, USA

Abstract

Aided by recent advances in computational power, algorithms, and higher fidelity data, increasingly detailed theoretical models of infection with influenza A virus are being developed. We review single scale models as they describe influenza infection from intracellular to global scales, and, in particular, we consider those models that capture details specific to influenza and can be used to link different scales. We discuss the few multiscale models of influenza infection that have been developed in this emerging field. In addition to discussing modeling approaches, we also survey biological data on influenza infection and transmission that is relevant for constructing influenza infection models. We envision that, in the future, multiscale models that capitalize on technical advances in experimental biology and high performance computing could be used to describe the large spatial scale epidemiology of influenza infection, evolution of the virus, and transmission between hosts more accurately.

Keywords

Influenza A virus model; Epidemiological; Viral kinetics; Networks; Metapopulation

1. Introduction

Interest in influenza A virus (IAV) infection has increased over the past several years, due to the emergence of highly pathogenic avian influenza A H5N1, avian influenza A H7N9, and zoonotic infections caused by these strains, the development of resistance to drugs currently available to treat influenza infection, and the occurrence of the most recent (H1N1 2009) human pandemic. As interest in this disease has increased, so, too, have the number of modeling studies focusing on it. Most work has focused on modeling influenza infection at a single scale, involving either infections at the tissue level in order to describe within-host viral dynamics (Smith and Perelson, 2011; Beauchemin and Handel, 2011; Smith and Ribeiro, 2010), or at the population scale in order to describe epidemiological findings (Ferguson et al., 2005, 2006; Germann et al., 2006; Halloran et al., 2008).

IAV dynamics occur over time scales ranging from hours (infection) to decades (evolution and immune response), and over spatial scales ranging from sub-micron (viral capsid) to tens of thousands of kilometers (geographical regions). Not all problems require multiscale modeling to address, but some interesting problems do.

*Corresponding author. Tel.: +1 505 667 6829; fax: +1 505 665 3493.

Recently, multiscale approaches have emerged that seek to couple population scale dynamics to events occurring within individual hosts. Multiscale modeling of influenza infection is made increasingly possible by a growing body of data available to constrain single scale models, and by the expanding availability of greater computing power. It is hoped that by including an increasing amount of detail in these models, over several scales, they will yield more accurate pictures of influenza outbreaks, epidemics, pandemics, and evolution.

Multiscale modeling of influenza infection could advance our ability to generate predictions of utility in both basic biology and public health. Applications include the study of IAV evolution and the optimization of antiviral drug use during small and large scale outbreaks. Multiscale modeling has the potential to yield predictive capabilities that are substantially more detailed than those provided by state-of-the-art single scale models.

In this review, we will discuss mathematical models of IAV infection at single scales, which form the basis for the generation of multiscale models, together with the small number of multi-scale models that have been published in this nascent field. Rather than provide an exhaustive overview, our goal is to provide a perspective on what has been accomplished in single scale models from the point of view of how models at each scale fit into the multiscale IAV modeling problem. We begin with an overview of IAV biology, focusing on data that is most relevant to modeling influenza infections within the human host. We then consider within-host influenza infection models, followed by population scale models. We examine how within-host models are coupled to population scale models, namely, through mapping within-host viral loads onto inter-host transmission parameters. With this framework in mind, we then discuss the multiscale models of influenza infection published to date. Finally, we offer an outlook for future work in this area of research.

2. Biology of influenza infection within the human host

2.1. Historical context

IAV has been studied extensively in vitro and in vivo since its discovery in swine by Shope (1931), although the virus was first isolated from poultry, as the causative agent of “fowl plague,” in 1901 (Centanni and Savonuzzi, 1901; Maggiora and Valenti, 1901; Lode and Gruber, 1901; Wilkinson and Waterson, 1975). IAV was first isolated from humans by Laidlaw and colleagues in 1933 (Smith et al., 1933). Green (1962) discovered how to grow influenza virus successfully in the laboratory in an immortalized cell line, demonstrating the serial propagation of the influenza B/Lee virus strain in Madin Darby canine kidney (MDCK) cells. This was a tremendous technical advance that, together with other improvements, allowed influenza strains to be propagated with ease in mammalian cells in the laboratory. Because of this accident of history and nature, and the rise of molecular biology, a large part of what we know today about influenza infections at the molecular level has been discovered in MDCK cells and several other laboratory cell lines. Similarly, much has been learned about influenza infection in the living mammalian host through experiments on mice and ferrets, and to a lesser extent guinea pigs, hamsters, cotton rats, horses, cats, pigs, non-human primates and humans (Tellier, 2006; Kroeze et al., 2012; Saenz et al., 2010). However, it is important to keep in mind that studies in neither MDCK cells in vitro nor non-human mammals in vivo completely captures human influenza disease. When modeling influenza infection in the human host, it is best to use data that most closely describes human disease.

2.2. IAV overview

Influenza viruses are negative strand RNA viruses. The IAV genome consists of eight gene segments encoding at least 12 proteins (Jagger et al., 2012). The virus is encapsulated in

host cell membrane, with three transmembrane virus proteins embedded in this membrane: hemagglutinin (HA), neuraminidase (NA), and the M2 ion channel. IAV binds target cells primarily through interactions between HA and terminal sialic acids on the surface of target cells. Newly budding virus is released from infected cells by the NA-mediated cleavage of sialic acid bound by the new virus particles. The M2 ion channel is required for delivery of virus particle contents into target cells; the virus particle is endocytosed, and within the endosome, at low pH, the virus envelope fuses with the endosomal membrane in an M2 dependent fashion to release the contents of the virus particle into the cell. The host cell machinery is then co-opted to produce viral proteins and to reproduce the viral genome. New virus particles are assembled at the cell membrane, bud out of the cell, and are released into the extracellular environment, lysing infected cells in the process (Palese and Shaw, 2007; Rossman and Lamb, 2011). However, until new virus particles are completely enclosed in an envelope of cell membrane and released, infected cells do not contain infectious virus particles, but rather only viral genes and proteins. Thus, while the budding and release of infectious influenza virus particles result in the lysis of infected cells, the lysis of infected cells by exogenous means does not result in the release of infectious virus particles. This feature of influenza virus is in contrast with other viruses, such as hepatitis C virus; in hepatitis C virus infection, cell lysis should lead to the release of fully formed, infectious virions harbored within the cell (Moradpour et al., 2007).

2.3. Morphology of IAV particles

Both a spherical and a “filamentous” morphology are seen for IAV particles. The filamentous particles could be described as flexible cylindrical rods, and are typically at least 500 nm long. Spherical particles are approximately 100 nm in diameter (Rossman and Lamb, 2011), with up to approximately 10% variability seen across strains. The diameter of the cross-section of filamentous particles is also approximately 100 nm. The length of filamentous particles can be up to more than 20 μm (Rossman and Lamb, 2011); the maximum and typical lengths of filamentous particles may vary by strain, with perhaps most strains having a maximum length of approximately 2–3 μm . Filamentous and spherical forms have both been observed to contain only one copy of the viral genome (Calder et al., 2010; Noda et al., 2006; Rossman et al., 2010; Hutchinson et al., 2010), and are comparably infectious (Roberts and Compans, 1998). Filamentous virus particles are hypothesized to be the primary agent of human IAV disease within the host, whereas spherical particles are proposed to play an important role in transmission between hosts (Roberts and Compans, 1998). Samples isolated from the human upper respiratory tract from in vivo human infections appear to contain primarily filamentous particles (Chu et al., 1949; Kilbourne and Murphy, 1960); filamentous particles were also found in lung sections from fatal 2009 pandemic H1N1 cases (Nakajima et al., 2010). In contrast, most laboratory adapted strains are predominantly spherical in shape. Early egg or cell culture passages of in vivo human isolates are predominantly filamentous, but with repeated passage in the laboratory, the spherical morphology becomes more prevalent. It is hypothesized that the small spherical variants grow more rapidly in laboratory conditions (Sieczkarski and Whittaker, 2005; Roberts and Compans, 1998). The ability to generate the filamentous morphology is under the control of at least two genetic loci, within the gene encoding the matrix protein (M1) and within the M2 gene, suggesting that both the matrix protein and the M2 ion channel play important roles in viral assembly and budding (Roberts et al., 1998; Rossman et al., 2010).

2.4. Infectivity of virus particles

Most IAV particles are not infectious. At low multiplicities of infection (MOIs) in embryonated eggs, approximately 0.4–4.1% of IAV particles produced are infectious (calculated from Table 1, Marcus et al., 2009); other estimates in eggs and mammalian cells suggest that from 0.2% to 1.0% of particles are infectious (Van Elden et al., 2001; Wei et al.,

2007). The percentage of virus particles that is infectious may vary across IAV strains, host cell types, and growth conditions, including in vivo vs. in vitro infection. At high MOIs, an even smaller percentage of IAV particles produced is infectious (von Magnus, 1954; Marcus et al., 2009), and across six strains studied, an average of only about 1 in 150 cells (primary chicken embryonic kidney) produced an infectious virus particle (Marcus et al., 2009). However, in addition to infectious particles, there are a variety of other biologically active particles, including defective interfering particles, noninfectious cell-killing particles, interferon-inducing particles, and interferon induction-suppressing particles (Marcus et al., 2009; Nayak et al., 1978; Huang and Baltimore, 1970).

Defective interfering particles contain a genome with at least one partial, internal gene deletion or other defect that causes at least one gene product to be missing (Marriott and Dimmock, 2010; Nayak et al., 1978; Huang and Baltimore, 1970). Defective interfering particles interfere with the production of infectious virus particles, and at higher MOIs, a greater proportion of virus particles produced are defective interfering particles (Marcus et al., 2009; Nayak et al., 1978; Huang and Baltimore, 1970). After 1–3 passages at a high MOI, the titer (effectively, the number per cell) of infectious particles produced declined by up to more than four orders of magnitude for one strain studied, even as the total titer produced stayed constant (Marcus et al., 2009). Even at low MOIs, however, the same study showed that the average ratio of the number of defective interfering particles to the number of plaque forming, infectious particles was 22, across six strains studied. Thus, local virus concentrations in tissues could have a profound impact on virus replication in vivo (Marcus et al., 2009); this could be an important consideration when modeling IAV infection on the tissue scale. It should be noted that defective interfering IAV particles have been found in natural chicken infections (Chambers and Webster, 1987); we are unaware of a comparable study of human IAV strains.

2.5. Receptor binding and endocytosis

In 1964, it became clear that sialic acid is the primary receptor for influenza viruses (Johnson et al., 1964). The HA of human-adapted strains preferentially binds α -2,6 linked sialic acids, while avian strains preferentially bind α -2,3-linked sialic acids (Suzuki, 2005; Matrosovich et al., 2004a; Thompson et al., 2006; Stevens et al., 2006). The IAV HA protein is significantly glycosylated, with the degree of glycosylation varying across strains, and these glycans affect HA binding to sialic acid (Wang et al., 2009). In addition to binding carbohydrates, IAV HA also binds to lectins on cells of the immune system. IAV is known to bind to the macrophage mannose receptor (MMR) via sialic acid dependent and independent mechanisms (Pontow et al., 1992; Reading et al., 2000), and binds to the macrophage galactose-type lectin (MGL) via a sialic acid independent mechanism (Upham et al., 2010). A human highly pathogenic H5N1 isolate and a human H3N2 strain have been shown to bind dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) (Wang et al., 2008; Londrigan et al., 2011), which is present on dendritic cells only (Svajger et al., 2010), and H3N2 binds liver/lymph node-specific intracellular adhesion molecules-3 grabbing non-integrin (L-SIGN) (Londrigan et al., 2011), which is found in the lung in a subset of alveolar epithelial cells and endothelial cells (Khoo et al., 2008). More glycosylated strains appear to be more efficient at infecting macrophages in vitro than less glycosylated ones (Reading et al., 2000). Finally, it should be noted that influenza viruses bind to sialic acids in mucus; this will be discussed in more detail in Section 2.10.

Upon binding to sialic acid moieties on respiratory epithelial cells, IAV is endocytosed. Endocytic receptors for IAV on respiratory epithelial cells are not known, although recent work suggests that the EGFR and potentially other receptor tyrosine kinases may facilitate IAV endocytosis (Eierhoff et al., 2010). Endocytosis of spherical IAV particles occurs by

clathrin dependent, caveolin dependent and clathrin and caveolin independent mechanisms, depending on the strain and host cell type (Lakadamyali et al., 2004; Nunes-Correia and Eulalio, 2004; Sieczkarski and Whittaker, 2005). Filamentous virus particles are too large to fit in an average clathrin coated pit (Sieczkarski and Whittaker, 2005); it has thus been hypothesized that filamentous IAV virions may enter cells by macropinocytosis, as do other filamentous viruses (Rossman and Lamb, 2011). Indeed, it has been shown that the internalization of filamentous influenza viruses into cells is delayed compared to that of spherical particles (Sieczkarski and Whittaker, 2005), consistent with macropinocytosis as a means of endocytosis for IAV, and IAV has recently been shown to be able to infect cells in vitro via a dynamin-independent mechanism that shares all features studied to date with macropinocytosis (de Vries et al., 2011).

2.6. Cleavage of HA for entry into the cytoplasm

The uncleaved HA protein, HA0, must be cleaved into two pieces, HA1 and HA2, in order for membrane fusion within the endosome or, perhaps, macropinosome, and hence successful infection, to occur (Palese and Shaw, 2007). Human adapted strains and nearly all low pathogenicity avian strains have a monobasic cleavage site in the HA0 protein that requires a trypsin-like protease for cleavage; some avian monobasic HA0 cleavage sites appear not to require such specific proteases and can instead be cleaved by ubiquitous enzymes such as furin (Pickens et al., 2010). High pathogenic avian strains have a polybasic cleavage site that can be cleaved by enzymes found ubiquitously, including furin. The specific enzymes used by human adapted IAV in the human respiratory tract in vivo are not known, but their selective localization within the respiratory tract is thought to be a major determinant of the respiratory tract tropism of human-adapted IAV. Several trypsin-like proteases (tryptase Clara, mast cell tryptase, mini-plasmin, ectopic anionic trypsin I, and TC30) have been isolated from rodent and swine lung tissues and can support viral replication in vitro (Kido et al., 1992; Chen et al., 2000; Murakami et al., 2001; Towatari et al., 2002; Sato et al., 2003). A human protease, human mast cell tryptase beta, homologous to two of these proteases, does not cleave IAV HA (Chen et al., 2000); thus, it should not be assumed that human homologues of these proteins are capable of cleaving HA. In particular, it is sometimes stated that tryptase Clara is likely to be the main human protease required to cleave HA, perhaps because it was the first such protease to be isolated from mammalian lung. However, tryptase Clara has so far only been purified directly from rat lung; we do not at present know the genetic sequence encoding this protein, and therefore do not know whether a homologous human protein even exists. Three trypsin-like proteases, TMPRSS2, TMPRSS4 and HAT, capable of cleaving human-adapted IAV HA, have been found in human airway tissues, including tissues higher in the respiratory tract (Bottcher et al., 2006; Bottcher-Friebertshauser et al., 2010; Bertram et al., 2010). It is not known whether cleavage of HA in vivo takes place intracellularly, extracellularly, or both (see, e.g., Bottcher-Friebertshauser et al., 2010); it is even possible that the localization of most or all functional cleavage proteases to specific cell types could be an additional factor facilitating IAV infection of such cell types and limiting infection of other cell types in vivo.

2.7. Number of infectious particles produced per cell

The number of infectious virus particles produced by a single infected cell varies with the infecting viral strain (Mitchell et al., 2011), the host cell type (human vs. avian, Mitchell et al., 2011, and different tissue origins within a host), MOI (Marriott and Dimmock, 2010) and growth conditions (e.g., temperature, as IAV is a temperature sensitive virus). Several investigators have made measurements in non-human culture systems. The work of Cairns and Edney suggests that egg cells infected with egg-adapted strains produced fewer than one infectious particle per cell (Cairns et al., 1952). Horsfall (1955) estimated that eggs inoculated with the influenza B/Lee strain at a low MOI produced peak measurable titers of

approximately 33–168 infectious particles per cell, and eggs inoculated with the PR8 strain produced 3–167 infectious particles per cell, with the production of infectious particles declining with increasing MOI; Horsfall noted, however, that infectious virus particles are rapidly inactivated in allantoic fluid. Stray and Air (2001) estimated that a single infected MDCK cell infected with either of three related influenza A strains produced 10^3 – 10^4 virus particles, based on converting from hemagglutination titer. The percentage of these particles that was infectious was not reported.

Virus production has been estimated based on theoretical modeling of data from one set of measurements of virus production in human air liquid interface (ALI) cultures of normal human bronchial epithelial (NHBE) cells, and is discussed in Section 3.2.1. ALI cultures of NHBEs closely mimic in vivo human lung tissue, and thus this study represents an important step towards a quantitative understanding of the replication of human influenza viruses in human tissues.

It should be noted that inoculation with both spherical and filamentous virus particles results in the appearance of newly assembling virions at the same time post-infection, as suggested by the appearance of cellular vRNP at the same time post-infection in Mv 1 Lu cells in vitro, using indirect immunofluorescence microscopy (Sieczkowski and Whittaker, 2005).

2.8. Infection, superinfection and viral evolution

One infectious virus particle is sufficient to initiate infection of one susceptible cell. The only caveat to this statement, in practice, is that viral titers are measured in a specific cell or animal system, and varying the target cell type or experimental conditions may render a particular virus stock more or less infectious. In in vivo human infections, it is not known how many infectious virus particles infect a given cell, or how many additional particles bind or enter a cell. We do know that nearly all human infections are with only one strain of IAV. However, mixed infections of an individual with more than one strain, and mixed infections within individual cells in one individual, do occasionally occur (Ghedini et al., 2009). Such superinfections with multiple strains can result in major evolutionary changes in the virus, through reassortment (i.e., bringing together new combinations) of genetic segments and recombination of portions of genetic segments. More frequently, however, IAV strains evolve by mutation, because the IAV polymerase has no proofreading activity (Palese and Shaw, 2007; Perelson et al., 2012), and hence the virus appears to replicate as a quasispecies (Gutierrez et al., in press; Llauro and Andino, 2010). Mutations frequently result in antigenic drift (Rambaut et al., 2008) and, less frequently, antigenic shift, which renders a majority of the population susceptible once again (see, e.g., Smith et al., 2004; Hensley et al., 2009; Wright et al., 2007).

2.9. Cells targeted by IAV

Studies of human post-mortem tissue from IAV infected patients reveal that human seasonal IAV primarily infects epithelial cells of the larger airways of the lower respiratory tract, whereas pandemic and zoonotic strains have a greater propensity to infect cells of the more distal lower respiratory tract as well, including alveolar epithelial cells, other lung cells, and infiltrating cells of the immune system, and also extrapulmonary tissues (Guarner and Falcon-Escobedo, 2009; Wright et al., 2007). Comparison of experimental, intranasal human infections with experimental, aerosol and natural human infections with seasonal human strains has revealed that infections initiated with aerosol inhalation, rather than intranasal inoculation, replicate the full spectrum of symptoms seen in natural infections (Tellier, 2006); this has been shown in ferrets as well (Gustin et al., 2011). Moreover, studies have shown that intranasal zanamivir does not prevent natural, seasonal IAV infection, whereas

inhaled zanamivir has been shown to be effective prophylactically (Tellier, 2006). Aerosol infection typically initiates infection in the lower respiratory tract (Tellier, 2006).

All IAV strains that infect humans are capable of infecting the respiratory epithelium from the nasal epithelium to the bronchioles, although seasonal strains rarely cause infections in bronchiolar or alveolar regions (Guarner and Falcon-Escobedo, 2009). In contrast, highly pathogenic avian influenza A(H5N1) infects type II pneumocytes in the alveolae, epithelial and other cells in the larger airways, and extrapulmonary tissues (Guarner and Falcon-Escobedo, 2009). With highly pathogenic strains, such as 1918–1919 H1N1 and highly pathogenic H5N1, or in severe cases of less pathogenic strains, alveolar pneumocytes and also intraalveolar macrophages tend to be infected (Guarner and Falcon-Escobedo, 2009). Inflammation, congestion and epithelial necrosis are seen in the larger airways with seasonal human strains and highly pathogenic strains; with highly pathogenic strains, inflammation is seen in the alveolae as well, resulting in diffuse alveolar damage, and hence often acute lung injury or acute respiratory distress syndrome, in susceptible individuals (Guarner and Falcon-Escobedo, 2009; Wright et al., 2007). Acute lung injury is typically accompanied by massive immune cell infiltration into the lungs and, in particular, alveolar regions (for quantitative mouse data, see Hashimoto et al., 2007; Perrone et al., 2008; Manicassamy et al., 2010).

Secondary bacterial pneumonia also frequently complicates severe cases and is an important cause of influenza related mortality (Kuiken and Taubenberger, 2008; Morens et al., 2008; Wright et al., 2007). IAV is thought to predispose to infection with *Streptococcus pneumoniae* by facilitating bacterial adherence to and growth in respiratory tissues and by causing defective immune responses (McCullers, 2006). It also predisposes to pneumonia due to *Staphylococcus aureus* infection as well (McCullers, 2006). Bacterial co-infection can also synergistically increase inflammatory responses, thereby causing greater harm, and can also exacerbate the primary viral infection (Iverson et al., 2011; Smith et al., submitted for publication).

When developing tissue scale models of viral infection, it is important to know the cell types that are infected by a viral strain of interest, the physical dimensions of the tissues involved, and how infections proceed within these tissues. This is critical for spatially explicit models, but is also important for estimating appropriate cell population sizes and other features of models that do not have a spatial component. Studies of IAV infections of ALI cultures of differentiated human airway epithelial cells show that IAV primarily binds the apical surface of target cells, corresponding to the luminal surface of the respiratory tract, and hence initiates infection from this surface (Slepishkin et al., 2001). The luminal surface of human respiratory tract epithelia, above the level of the alveolae, consists primarily of ciliated cells and non-ciliated, secretory cells, forming a polarized, pseudostratified, columnar epithelium (Kuhn and Wright, 2005). Additional studies of IAV infection of ALI cultures of human airway cells have revealed that human-adapted strains infect non-ciliated cells almost exclusively, whereas avian strains predominantly infect ciliated cells (see Figs. 1 and 2). This difference corresponds to a predominance of α -2,6 linked sialic acids on the apical surfaces of non-ciliated cells, compared to α -2,3 linked sialic acids on ciliated cells (Matrosovich et al., 2004a; Thompson et al., 2006). It is interesting to note that a 1968 pandemic-era strain infected more ciliated cells than do typical human seasonal strains, thereby demonstrating incomplete adaptation to humans (Thompson et al., 2006). The different cell populations targeted by human, avian and also swine strains should be taken into consideration when designing tissue scale models of human vs. avian or swine IAV infection.

The physical dimensions of the tissues involved in IAV infection are important to know when designing a spatial tissue scale model. The bronchial epithelial layer, which includes ciliated cells and non-ciliated secretory cells that together form the luminal surface, plus basal cells resting on a basement membrane, is approximately 21–28 μm thick in healthy individuals (Innes et al., 2006; Cohen et al., 2011). Ciliated cells are approximately 3–25 times more numerous than secretory cells in the large bronchi. Ciliated cells in the large bronchus are up to 20 μm thick (in the direction perpendicular to the luminal surface) and 10 μm in apical surface diameter, and have approximately 200 cilia on the apical surface, 3–6 μm long each; bronchial cilia have elsewhere been estimated to be 5–8 μm long and 0.3 μm in diameter (Kuhn and Wright, 2005; Tomashefski et al., 2008). In the bronchi, the secretory cells are predominantly goblet cells, which have bulging apical surfaces covered by microvilli. Small mucous granule cells (SMGCs) are an additional, rare secretory cell type in the bronchi, and may actually be morphological variants of goblet cells (Kuhn and Wright, 2005). Normal human bronchi have approximately 42,000 goblet cells per mm^3 of tissue (Rogers, 2003; Innes et al., 2006; Ordonez et al., 2001), and 873 ± 93 goblet cells per mm^2 surface area of basal lamina (Innes et al., 2006). The apical surface area of goblet cells is comparable to that of ciliated cells. In one study, in ALI cultures of normal human tracheal cells, goblet cells comprised 8% (352 ± 9) of all cells (4227 ± 310) in apical fields of view ($320 \mu\text{m} \times 320 \mu\text{m}$), and non-ciliated membranes (which almost entirely belong to goblet cells) accounted for $27.43\% \pm 5.48\%$ of the total apical membrane surface area (Lachowicz-Scroggins et al., 2010), giving an apical surface area of approximately $79.8 \mu\text{m}^2$ per cell, or an average apical surface diameter of 10:1 μm . Goblet cells secrete mucus, and mucus is also secreted from mucous glands lying in the subepithelial compartment of the bronchi. The frequency of appearance of these glands decreases distally in the bronchial tree; mucous glands are not typically found in bronchioles. The secretory cells in the bronchioles are Clara cells, which do not secrete mucus, but instead contribute several proteins, including surfactant apoproteins A, B and D, to the surfactant film lining the bronchioles (Kuhn and Wright, 2005). Clara cells have an apical surface diameter of 0.3–0.8 μm (Kuhn and Wright, 2005).

2.10. Interaction of mucus with IAV

Mucus acts as a first defense barrier for the respiratory tract. It consists of a periciliary, watery, serous layer, in which cilia beat, beneath a viscous mucous layer. It is pushed upward and out of the respiratory tract by cilia, and its rate of movement varies with level in the bronchial tree; mucus moves fastest in the trachea and central bronchi (1–2 cm/min), and slower in peripheral bronchi (Kuhn and Wright, 2005; Tomashefski et al., 2008). Airway mucus is rich in α -2;3 linked sialic acids (Lamblin et al., 1984; Breg et al., 1987), and human bronchial mucins potentially inhibit binding of avian IAV strains recognizing α -2;3 linked sialic acids, but not human adapted strains recognizing α -2;6 linked sialic acids (Couceiro et al., 1993). Indeed, it is speculated that a variety of selection pressures in the respiratory tract, including sialic acid moieties on epithelial cells and in mucus, have resulted in the evolution of strongly preferential binding of HA to α -2;6 linked sialic acids, and thus infection of non-ciliated cells, by human adapted IAV strains (Couceiro et al., 1993). Moreover, the presence of mucus, rich in α -2;3 linked sialic acids, may be one feature of the human host which renders direct adaptation of avian IAV strains to human hosts evolutionarily challenging. In ALI culture models, mucus is typically removed from cells before inoculation with IAV (see, e.g., Matrosovich et al., 2004a); thus, work in ALI cultures of airway epithelial cells may suggest an artificially high propensity of avian viruses to infect such cells, compared to in vivo host conditions. However, mucus does bind human adapted IAV strains as well, and this binding is abrogated by IAV NA activity; an NA inhibitor potentially inhibits infection of ALI cultures through mucus, and suggests the importance of the IAV NA protein in initiating infection in vivo (Matrosovich et al., 2004b).

2.11. Innate and adaptive immune responses

Upon infection with IAV, cells activate signaling cascades to effect intracellular changes that minimize virus replication, and to release mediators of innate immunity into the extracellular environment. Principal among these extracellular mediators are the type I interferons (IFNs), which induce a protective, antiviral state in uninfected cells, and a central function of the IAV protein NS1 is to suppress the IFN response (Wright et al., 2007; Garcia-Sastre, 2011). IAV infection also activates the inflammasome and triggers both innate and adaptive immune responses that are inflammasome-dependent (Pang and Iwasaki, 2011). In the adaptive immune response to IAV infection, both CD8⁺ T cells and primarily CD4⁺ T cell dependent antibody production are instrumental in viral clearance and the development of immunity (Wright et al., 2007; Kreijtz et al., 2011; Valkenburg et al., 2011). Antibodies directed against the IAV HA and NA surface antigens are of particular importance, and a subset of such antibodies, called neutralizing antibodies, develops that is directed against the HA of a specific strain and is capable of blocking virus entry into cells and hence of preventing infection (Kreijtz et al., 2011; Valkenburg et al., 2011). CD8⁺ T cells help to clear IAV infections by killing infected cells and releasing proinflammatory cytokines (Valkenburg et al., 2011). During infection, antiviral and inflammatory cytokines, and chemokines are released locally and systemically; interleukin-6 (IL-6) and IFN- α are induced in respiratory tract secretions in experimentally infected humans, and, among many elevated cytokines, their levels are most correlated with the severity of clinical symptoms (Hayden et al., 1998; Wright et al., 2007). Viral titers peak approximately 48 h after infection, and decline thereafter over several days (Murphy et al., 1973; Douglas, 1975; Richman et al., 1976; Carrat et al., 2008; Canini and Carrat, 2011). A gene expression study of peripheral blood in human experimental IAV infection revealed that systemic host immune responses differ between symptomatic and asymptomatic infected individuals (Huang et al., 2011). The host immune response is required for clearing the IAV infection; nonetheless, host responses contribute to the pathology seen particularly with highly pathogenic strains (Fukuyama and Kawaoka, 2011).

Following infection with a given strain, sufficiently high antibody titers against that strain, and hence immunity to that strain, can be lifelong (Valkenburg et al., 2011). A small pool of antigen-specific memory CD8⁺ T cells contributes to this protection, and offers some level of protection against heterologous IAV strains as well (Valkenburg et al., 2011). Some level of immunity can spill over to antigenically similar strains, and antigenic drift is thought to be driven by this immune pressure (Valkenburg et al., 2011).

2.12. Spatial aspects of IAV infection

IAV infections spread inhomogeneously within the lung. Little is known about the spread of IAV infection at the tissue scale; however, photographs of ALI cultures of NHBs infected with IAV clearly show spatial inhomogeneities, and, moreover, the preferential spread of infection from cells to their nearest neighbors, within 24 h (see Fig. 2; Matrosovich et al., 2004a; Thompson et al., 2006). Such clustering on the tissue scale can be seen in fatal human cases as well (see, e.g., Monsalvo et al., 2011; Piwpankaew et al., 2010). Whether such clustering results from direct cell-to-cell transmission or from free virus released from one cell preferentially infecting neighboring cells is not known.

Pathological findings in fatal human cases are also typically patchy at the scale of the lung (Guarner and Falcon-Escobedo, 2009). A study of experimental infections of horses quantified this patchy distribution across bronchioles; a maximum of approximately 5% of mucosal bronchiolar epithelial cells was infected at any one time, with the peak estimated to be at day 4 post-infection (Saenz et al., 2010). A recent landmark study of mouse infection with green fluorescent protein (GFP)-tagged IAV PR/8 also showed the focal nature of

infection with in vivo, whole-lung imaging over time (Manicassamy et al., 2010). Histopathology in ferrets also shows focal lesions (Guarner and Falcon-Escobedo, 2009), as do studies of infections in macaques with 1918–1919 H1N1, highly pathogenic H5N1 and seasonal human strains (Rimmelzwaan et al., 2001; Kobasa et al., 2007; Baskin et al., 2009; Cilloniz et al., 2009).

2.13. Tissue temperature

An additional important consideration with IAV infection is tissue temperature. Most human-adapted strains are generally capable of growth at temperatures encountered within the human respiratory tract, which range from approximately 30.2 °C to 37.5 °C normally (McFadden et al., 1985; Kohl, 1990; Lindemann et al., 2002), and up to 41 °C during infection (Douglas, 1975; Nicholson, 1992). However, changes in temperature within this range can produce large changes in growth kinetics, depending on the strain (see, e.g., Sugiura et al., 1973), and especially as temperatures reach 40 °C, at which point the production of infectious particles has been shown to diminish for some strains (Overton et al., 1986). Moreover, strains that are not human-adapted often do not grow well at temperatures typically encountered in the human upper respiratory tract (Massin et al., 2010). Avian influenza strains are adapted to grow well at 40 °C, and do not grow well at 33 °C, at which temperature human strains replicate well (Massin et al., 2010). Swine strains grow well in the swine respiratory tract at 37 °C, and their growth at greater or lower temperatures varies with IAV strain and host cell type (Massin et al., 2010). The PB2 and PA subunits of the IAV polymerase complex are known to play an important role in temperature sensitivity of the virus (Kashiwagi et al., 2010).

Thus, when modeling human infection with zoonotic strains, it is important to consider temperatures encountered in the human respiratory tract, as these temperatures contribute to determining the locations of successful infection within the respiratory tract and the relative extent of viral replication and shedding across locations. There is a temperature gradient in the airway, with temperatures ranging from the external temperature outside the mouth and nose to the core body temperature, ~37° C, within alveolae. The temperature within human airways varies with the temperature and humidity of inspired air, and with inspiration and expiration (Kohl, 1990; McFadden et al., 1985). The nasal mucosal temperature varies across locations within the nose from 30.27 ±1.7 °C to 34.47±1.1 °C (Lindemann et al., 2002). The mucosal temperature at the carina, at the bottom of the trachea where the trachea branches, during inspiration of room temperature air (average 20.2 °C), is 34.2 °C on average, with some inter-individual variability; the mucosal temperature is 35.1 °C on average in the bronchus intermedius, and 36.4 °C on average in more distant bronchi, between the ninth and tenth bifurcation (B9) (Kohl, 1990).

It should be noted that these temperatures are at mucosal surfaces, whereas virus particles encounter temperatures at cell surfaces (relevant for binding and fusion) and within cells (relevant for replication). We do not know of any temperature measurements at the apical surface of respiratory tract epithelial cells, or within these cells. However, it is likely that the temperature at the mucosal surface is at least within 1 °C of the temperature experienced by IAV virions. The thickness of the luminal surface, from the top of the mucus layer to the bottom of infected epithelial cells, is typically a maximum of 50 µm. There would need to be a temperature gradient of 20 °C/mm at the mucosal surface in order for there to be a 1 °C difference across 50 µm, and we find this unlikely given the high water content of the lung epithelia and mucous layers (i.e., the relative lack of a thermal insulator) and the relative lack of a highly thermogenic source (arterioles, muscle, or brown fat) at the luminal surface.

2.14. Human transmission

Influenza transmission from one individual to another is influenced by factors within the infected individual, within the susceptible host, and in the environment. Within an infected individual, the stage of illness and inter-individual variability in severity of illness and types of symptoms influence the degree and methods of viral shedding. Within the susceptible host, the nutritional status, overall state of health and prior exposure to antigenically related strains affect susceptibility. In the environment, absolute humidity and temperature have been shown to affect the transmissibility of virus particles. Additional factors affecting the probability of transmission due to a single contact include the degree of proximity of the infected and susceptible individuals during that contact, the duration of the contact, and the precise nature of the contact, i.e., whether virus particles are introduced into the vicinity of the host as large or small particles in the air, as fomites deposited on host mucous membranes or environmental surfaces, or some combination of the above.

The single most important factor governing transmission is the stage of illness of the infected individual. Viral shedding in adult human volunteers peaks on day 2 and lasts an average of 4.8 days (Carrat et al., 2008); the duration of shedding varies with IAV strain (see, e.g., Ng et al., 2010). On average, viral shedding begins during the first day after infection, before symptoms appear, and can be detected on day 1 in 83% of study subjects, on day 2 in 14% of subjects, and day 3 in 3% of subjects. It is important to note that in these human experiments, only 66.9% of study subjects, with a 95% confidence interval of [58.3%, 74.5%], developed symptomatic infections, which is consistent with epidemiological observations of natural influenza disease. Furthermore, asymptotically infected patients contribute significantly to viral shedding during an outbreak, as between 83.9% and 92.8% of subjects experimentally infected with IAV, far greater than the percentage that develops symptoms, detectably shed virus, depending on the strain studied. (See Carrat et al., 2008 for further quantitative results.)

Inter-individual variability in disease course and viral shedding can be caused by age differences, nutritional status, health status, and undoubtedly other, as yet unknown factors. Shedding in children (Li et al., 2010; To et al., 2010; Bhattarai et al., 2011; Esposito et al., 2011) and hospitalized adults (Li et al., 2010; Malato et al., 2011; Meschi et al., 2011) can last longer than in previously healthy adults with mild clinical courses. Pregnant women, young children and the elderly are at greater risk of severe disease, as are immunocompromised individuals and people with certain chronic health conditions, including asthma, chronic obstructive pulmonary disease, cardiovascular disease, obesity and diabetes (Medina and Garcia-Sastre, 2011). Mice whose diets are deficient in selenium (Beck et al., 2001), and individuals who are exposed to arsenic (Kozul et al., 2009; Liao et al., 2011) are known to have more severe IAV disease. Persons with low serum vitamin D levels report more severe upper respiratory tract infections (Cannell et al., 2008).

A variety of factors are also thought to alter susceptibility to IAV infection in humans. Malnutrition and chronic disease, as are often seen in developing countries, greatly impact susceptibility. Individuals with low serum vitamin D levels report 40% more upper respiratory tract infections, compared to individuals with normal vitamin D levels (Ginde et al., 2009; Cannell et al., 2008). Most importantly, prior exposure to strains of IAV within an antigenic cluster typically prevents subsequent infection with a strain from the same cluster; IAV H3N2 clusters lasted an average of 3.3 ± 1.9 years from 1969 to 2003 (Smith et al., 2004).

Transmission occurs at different rates by direct contact, indirect contact, or deposition of large ($> 5 \mu\text{m}$ diameter) or small ($< 5 \mu\text{m}$ diameter) droplets onto mucosal surfaces, typically within the respiratory tract (Brankston et al., 2007). Experimentally, infectious dosages of

influenza viruses are often measured in units of a titration of a virus sample that would infect 50% of a set of target cell samples; when the target cells are cultured in the laboratory, this unit is called the tissue culture 50% infectious dose, or TCID₅₀. Experimental aerosol infections of humans with no detectable neutralizing antibodies with approximately 1, 2, and 5 TCID₅₀, as assayed in primary rhesus monkey kidney tissue culture, resulted in symptomatic infections at the doses of 1 and 5 TCID₅₀; 4 out of 14 total study subjects with low or no detectable neutralizing antibodies developed symptomatic disease at those doses (Alford et al., 1966). If one hypothesizes that 60% of inhaled particles are retained in the respiratory tract, then this study would suggest that approximately 0.6–3 TCID₅₀, approximately equivalent to 0.4–2.1 plaque-forming units (pfu, our conversion, assuming approximately 0.7 pfu per TCID₅₀), are sufficient to initiate infection by aerosol inhalation (Alford et al., 1966; Tellier, 2006). In contrast, a separate study, using a different human seasonal strain and TCID₅₀ assay, showed that 127–320 TCID₅₀, equivalent to 89–224 pfu (our conversion), are required to initiate infection by nasal drops (Douglas, 1975; Tellier, 2006).

Humidity and temperature both affect the survival of influenza particles in the environment. A study in guinea pigs showed that infectious IAV particles are shed and detectable for nearly 2 days longer when the ambient temperature is 5 °C, rather than 20 °C, and that transmission declines with increasing relative humidity and increasing temperature (Lowen et al., 2007). Analogous findings at the epidemiological scale have been made recently, with the discovery of a correlation between influenza outbreaks and absolute humidity (Shaman et al., 2006), which appear to have a stronger correlation than other climatological variables. This important work, however, was carried out for the United States only, and questions remain about transmission in other geographical regions, such as the tropics (Viboud et al., 2006). These findings have also shed significant light on the causes of the seasonality of influenza in temperate regions of the globe; nonetheless, the causes of influenza seasonality are not yet fully understood (Lipsitch and Viboud, 2009).

2.15. Limitations of experimental data

One final and obvious consideration is that experimental data is inherently noisy. Aside from measurement errors intrinsic to any experimental process, results may not be completely comparable across researchers, and the results from one laboratory may not be completely comparable across or even within studies. To give one example of how data may not be comparable even within a given study, the infectivity of virus suspensions decays with time (De Flora and Badolati, 1973b; Beauchemin et al., 2008), increasing temperature (De Flora and Badolati, 1973b) and freeze-thaw cycles (De Flora and Badolati, 1973a), but when virus titers are reported in the literature, it is often not stated whether virus suspensions were titered under the same conditions as those under which experiments were performed, or whether suspensions were titered after an additional freeze-thaw cycle. Moreover, different studies often involve different experimental systems; results can vary substantially with the strain studied, MOI used, and cell type or animal host studied, among other variables. Finally, most experiments are not conducted with a quantitative focus. As such, data may lack important time points, or be at best semi-quantitative in important ways. These points are important to keep in mind when evaluating experimental data for use in modeling studies.

3. Within-host influenza infection models

Within-host models of IAV aim to discover features of viral infection that are difficult to determine directly by experiments. Such models describe IAV infection at the tissue scale, i.e., at the multicellular scale (Smith and Perelson, 2011; Beauchemin and Handel, 2011; Smith and Ribeiro, 2010). A small number of models have also been generated to describe

IAV infection within individual cells (Hatada et al., 1989; Sidorenko and Reichl, 2004; Sidorenko et al., 2008; Heldt et al., 2012). IAV binding and endocytosis in cell culture has also been modeled (Nunes-Correia et al., 1999). However, to date, there is insufficient published experimental data, in the form of either viral gene expression or protein levels, for very complete intracellular models to be constrained and made biologically relevant. Thus, within-host, tissue-scale models of IAV infection typically treat the cell as a “black box,” which can become infected and from which virus particles emerge. The main goal of within-host models is then to describe within-host viral kinetic data as accurately as possible. Features of IAV infection that within-host models have been used to estimate include the lifespan of an infected cell, the percentage of target cells that become infected over the course of infection, the number of infectious virus particles released per cell, the half-life of infectious virus particles (i.e., the half-life of their infectivity), and the within-host basic reproductive number, R_0 (Mohler et al., 2005; Baccam et al., 2006; Saenz et al., 2010; Miao et al., 2010a; Mitchell et al., 2011; Smith et al., 2011; Pawelek et al., 2012). Additionally, within-host models can be used to discover a relationship between viral kinetic data and symptom dynamics (Canini and Carrat, 2011).

A variety of approaches have been taken to model IAV infection within the host (Smith and Perelson, 2011; Beauchemin and Handel, 2011; Smith and Ribeiro, 2010). Ordinary differential equations (ODEs), cellular automata, individual based approaches, and partial differential equations (PDEs) have been used. Because there is often a small number of data points available to constrain model parameters, these models tend to be made as simple as possible; parameter identifiability is an important issue to keep in mind when evaluating or developing within-host IAV models. Some models hypothesize that the number of target cells is the limiting factor for virus growth (Baccam et al., 2006), and exclude both innate and adaptive immune responses, while others include immune responses (Bocharov and Romanyukha, 1994; Hancioglu et al., 2007; Handel et al., 2007; Lee et al., 2009; Miao et al., 2010a; Handel et al., 2010; Saenz et al., 2010; Pawelek et al., 2012). It is worth mentioning that, in the context of other, non-IAV viral infections, models of viral infections have been developed that include both intracellular and extracellular components (e.g., Haseltine et al., 2005; Guedj and Neumann, 2010; Dahari et al., 2011; Guedj et al., 2013).

3.1. Modeling mild vs. severe infections

All within-host models to date focus on epithelial cells as target cells. This focus, in turn, implies a focus on modeling mild human infection with seasonal or mild pandemic influenza strains, in which immune system cell infiltration is probably limited and epithelial cells are, indeed, the primary target cell type (Guarner et al., 2000). When modeling mouse data, this approach can be warranted, as well; human seasonal influenza strains tend not to replicate well or at all in mouse cell types other than respiratory epithelia (see, e.g., Perrone et al., 2008). However, highly pathogenic strains, including 1918–1919 H1N1 and highly pathogenic avian influenza (HPAI) H5N1, do replicate in non-epithelial tissues, both within and outside the lung, in humans and in all animal species studied to date (see, e.g., Kuiken et al., 2003; Perrone et al., 2008; Guarner and Falcon-Escobedo, 2009; Piwpankaew et al., 2010). Because different tissue types can exhibit different infection kinetics, each target cell type would most likely merit individual treatment in order to describe infection with highly pathogenic strains most accurately. For instance, highly pathogenic H5N1 infects lung microvascular endothelial cells, and the involvement of endothelial cells is suspected to play an important role in the clinical course of severe disease (Ocana-Macchi et al., 2009; Chan et al., 2009; Thitithanyanont et al., 2010; Viemann et al., 2011; Zeng et al., 2012). Similarly, highly pathogenic IAV strains productively infect macrophages (i.e., these strains infect macrophages and cause the production and release of infectious viral particles) during mouse infections in vivo (Perrone et al., 2008), and infect human and macaque macrophages

in vivo (Kuiken et al., 2003; Guarner et al., 2000; Gao et al., 2010), as does 2009 pandemic H1N1 (Takiyama et al., 2010). Highly pathogenic and human-adapted strains productively infect human macrophages in vitro (Perrone et al., 2008), and are therefore thought to productively infect human macrophages during in vivo infections (La Gruta et al., 2007), as well; it has also only recently been recognized that human macrophages can produce substantial viral titers when infected with human and highly pathogenic IAV strains (Perrone et al., 2008). Moreover, macrophages infiltrating the lung during infection with a highly pathogenic IAV strain greatly outnumber target epithelial cells (for quantitative mouse data, see Perrone et al., 2008; Hashimoto et al., 2007). Thus, ignoring infection of macrophages in a model of highly pathogenic IAV infection could yield inaccurate results, and applying currently existing within-host models to infections with highly pathogenic strains could yield unphysiological results. Other potential target cell types could be considered as well.

One model has attempted to address the role that a second target cell type could play in severe human IAV disease (Dobrovolny et al., 2010). In this two target cell model, the target cell types chosen are ciliated and non-ciliated epithelial cells of the respiratory tract. While this model was able to explain mouse infection data with more biologically realistic parameter values, it did not provide a better statistical fit to severe human H5N1 infection data than did a single target cell model. One difficulty, as always, is that the more complex two target cell model requires more parameters to fit, and data points are scarce. Nonetheless, this model takes an important step forward towards describing severe IAV disease by examining the involvement of more than one target cell type. For the future, it would be worthwhile to determine whether models that include additional or different target cell types may be able to describe severe human IAV disease, such as seen with H5N1 infection, with improved statistical fits and even more biologically realistic parameter values.

3.2. Target-cell limitation models

Baccam et al. (2006) developed a set of within-host, target-cell limited models of IAV infection using ODEs. The models were fit to viral kinetic data from nasal washes in experimental human IAV infection, in order to estimate parameters, and were of increasing complexity, with the most complicated model incorporating both a delay in viral production and an innate immune response. One of their models, shown in Fig. 3, which includes a delay in viral production but not an immune response, is

$$\frac{dT(t)}{dt} = -bT(t)V(t) \quad \frac{dI_e(t)}{dt} = bT(t)V(t) - gI_e(t) \quad \frac{dI_i(t)}{dt} = gI_e(t) - \delta I_i(t) \quad \frac{dV(t)}{dt} = pI_i(t) - cV(t). \quad (1)$$

This model has five parameters. The rate of infection of target cells, $T(t)$, by virions, $V(t)$, is captured by b . Target cells, when first infected, do not produce new virions; this phase is referred to as the eclipse phase, with population density $I_e(t)$. At a mean rate g , the eclipse phase cells begin producing virus and are relabeled as $I_i(t)$, which produce virions, $V(t)$, at a mean rate p per cell. These infected cells die at mean rate δ , and virions are generically cleared at mean rate c . This model successfully describes viral kinetic data. Models of this type predict that a viral infection ceases when the reservoir of target cells is depleted; for this reason, such models are referred to as “target cell limited” (TCL).

While simpler models that do not include the eclipse phase also fit the data well, models that include the eclipse phase result in estimated parameter values that may be in more biologically realistic ranges (Baccam et al., 2006; Beauchemin and Handel, 2011; Holder and Beauchemin, 2011). It is important to note, however, a major difficulty that arises when only viral titer data is available. Viral titers rise and fall approximately exponentially, a

behavior that only requires four parameters to describe: on a log-linear scale the viral levels are two intersecting lines to a very good approximation. Worse, this behavior also implies that assumptions within even the simplest models are suspect. For example, in (1) there are five parameters *and* a hidden assumption of exponentially distributed delays in each of the processes. Recently, Holder and Beauchemin (2011) consider several delay distributions within an otherwise fixed model and find important differences in the fitted values of the parameters.

3.2.1. Estimating the number of virus particles produced per cell—In one study, by fitting both a standard target-cell limited model using ODEs and a target-cell limited cellular automaton model to virus titer data, the rate of production of infectious virus particles released from ALI cultures of NHBE cells was estimated to be 0.71–1.4 pfu/hour (pfu/h) for a human seasonal strain inoculated at an MOI of 0.01, 18.3–19.0 pfu/h for 2009 pandemic H1N1 inoculated with an MOI of 0.01 or 0.001, and 0.20 pfu/h for an avian strain inoculated with an MOI of 0.01 (H5N1, fatal human case; Mitchell et al., 2011). The infected NHBE cell lifespan is estimated to be approximately 24 h, with an estimated range of 22–29 h, based on modeling of NHBE data from this study and of *in vivo* equine and murine data, and depending on the inoculating strain (Mitchell et al., 2011; Saenz et al., 2010; Miao et al., 2010a). The duration of the eclipse phase, i.e., the length of time between when a viral inoculum is first added to cultures and when infectious virions can first be measured in cell culture media, in ALI cultures of NHBEs, is approximately 8–10 h after infection (Mitchell et al., 2011). Comparable eclipse phase durations are seen in human macrophages *in vitro*, again varying by strain (Perrone et al., 2008; Mok et al., 2010), although the infected cell lifespan may be different from that of NHBEs. Also, infected epithelial cells in the human host die primarily by necrosis, whereas infected macrophages die by apoptosis (Mok et al., 2010; Hofmann et al., 1997).

It should be noted that the results of the Mitchell et al. (2011) study are inherently uncertain, due to issues of parameter identifiability and inherent limitations of the data (see, e.g., Miao et al., 2010b; Holder and Beauchemin, 2011). Again, however, because these estimates are calculated using data generated using ALI cultures of NHBEs, they represent the closest existing estimates to the human *in vivo* case. It should also be pointed out that these cultures do not include more than the most basic innate immune response, and as such do not take into account the full host immune response, including fever and viral clearance; the full immune response can reduce both viral production rates and infected cell lifespans.

Using results from the Mitchell et al. (2011) study, the total number of infectious virus particles produced by an individual, infected NHBE cell can be roughly estimated to lie between $(0.71 \text{ pfu/h}) \times [(24 \text{ h lifespan}) - (10 \text{ h eclipse phase})] = 10 \text{ pfu}$, as a lower bound, and $(1.4 \text{ pfu/h}) \times [(24 \text{ h lifespan}) - (8 \text{ h eclipse phase})] = 22 \text{ pfu}$, as an upper bound, i.e., 10–20 pfu, for a human seasonal strain; similarly, 260–300 pfu for the 2009 pandemic H1N1 strain; and 2–3 pfu for highly pathogenic H5N1. Thus, we can estimate that at least one strain well adapted to humans may produce approximately 10–20 infectious virus particles (pfu) per infected human respiratory tract cell; the recent pandemic strain may produce approximately an order of magnitude more infectious virus particles per infected cell; and a poorly adapted zoonotic strain may produce an order of magnitude fewer such particles per infected cell. Whether this relationship holds generally, among a majority of strains, remains to be determined. In general agreement with these results, others have estimated that mouse-adapted IAV PR8 produces 28 pfu per infected mouse cell *in vivo* (Smith et al., 2011).

If we assume, conservatively, that as few as 0.1–0.2% of virus particles produced by an individual cell are infectious, then we further estimate that each human respiratory tract cell infected with a human-adapted strain produces on the order of 5000–10,000 virus particles.

This calculation is consistent with the previous work in which it was estimated that a single MDCK cell infected with an equine IAV H3N8 strain produces a total of nearly 20,000 virions (Mohler et al., 2005).

While the work of Mitchell et al. examined IAV replication in realistic cultures of NHBE cells, other studies of viral replication have examined data collected in other cell culture systems and from human infections. Baccam et al. (2006) estimated that a single infected cell infects approximately 22 other cells—and hence produces at least 22 infectious virus particles. Handel et al. (2007), studying two nasal wash data sets from human experimental infections, estimated that infectious virus particles were produced at a rate of $1:1-1:2 \times 10^{-5}$ TCID₅₀=d ml, and offered a very approximate conversion factor of 1 TCID₅₀=ml of nasal wash per approximately 10^2-10^5 infectious virions at the site of infection within the respiratory tract. Holder and Beauchemin (2011) have also modeled both in vitro and human experimental data to estimate viral production rates in vitro and peak viral loads in humans in vivo. Cowling et al. (2010) measured the number of viral particles and TCID₅₀ per nasal and throat swab in humans over days after natural infections with both 2009 pandemic and seasonal IAV; they estimated the peak means of these measurements to be approximately 10^6 copies/ml and $10^{3.5}$ log₁₀ TCID₅₀, respectively.

3.3. Inclusion of an immune response

Including the immune response in the model more mechanistically than through the clearance rate c can be more biologically realistic, as we know that the immune response is ultimately responsible for clearing virus and ending IAV infection. Types of immune responses considered include innate immune responses, in the form of IFN signaling and NK and phagocytic cell activities; CD8⁺ and CD4⁺ T cell mediated responses; and antibody responses.

Models that include IFN signaling have hypothesized that IFN reduces the viral production rate by cells that are already infected, decreases the probability of a cell becoming infected (Baccam et al., 2006), or allows a cell to become refractory to infection (Saenz et al., 2010; Pawelek et al., 2012). Models that include no spatial information, such as ODE models, cannot distinguish cells adjacent to infected cells from cells distant from infected cells, and thus imply that IFN protects all cells instantaneously. One IAV model that includes spatial information is a cellular automaton model that hypothesizes that both virus and “a single diffusible antiviral factor” released from infected epithelial cells diffuse in two dimensions (Mitchell et al., 2011). This model could be expanded to take into account that infection appears to spread primarily to immediately adjacent cells, as both air–liquid interface in vitro and post-mortem histopathology data, discussed in the previous section, show clusters of immediately adjacent cells that are infected, with large uninfected areas between clusters (Matrosovich et al., 2004a; Thompson et al., 2006; Monsalvo et al., 2011; Piwpankaew et al., 2010). It is possible that the time from infection of a given cell to the release of new virus progeny is less than the time to IFN release and the subsequent induction of an antiviral state in a neighboring cell; this could favor the infection of closer target cells and the protection of more distant cells. Spatial models of IFN signaling in other viral infections have been constructed, some of which propose protection from infection specifically at sites distant from infected cells (Lam et al., 2005; Howat et al., 2006; Getto et al., 2008; Haseltine et al., 2008). In addition, as has been proposed by Roberts and Compans (1998), the production of long, filamentous virus particles in vivo could favor binding to and initiating infection of adjacent cells even before viral release. Moreover, in vivo, mucociliary clearance mechanisms may greatly hinder infection of all but adjacent cells (Roberts and Compans, 1998).

One recent study that examined the human virus-specific CD8⁺ T cell response in people infected with the 2009 pandemic virus revealed an early CD8⁺ T cell recall response that peaked within one week of infection and then rapidly contracted (Hillaire et al., 2011). Previously, only the most complex theoretical models have examined CD8⁺ T cell responses, and these models have been based on little data. One such complex model of CD8⁺ T cell dynamics in influenza infected mice showed that the spleen, rather than the draining lymph nodes, contributed the most CD8⁺ T cells to the lung during IAV infection (Wu et al., 2011). This study further showed that CD8⁺ T cell dynamics were more closely correlated with the numbers and kinetics of professional antigen presenting cells than with viral load.

A computer simulation model of the within-host antibody response to IAV infection that included within each host a repertoire of 10^7 B cells and that accounted for affinity maturation was developed by Smith et al. (1999). By representing B cell surface receptors, antibodies, and viral epitopes by strings of symbols and using a string matching algorithm, the antigenic distance between viral strains could be defined. Further, the same distance measure could be used between an antibody and a viral strain and allowed one to define the affinity of the antibody for that virus. The model, which could reproduce the dynamics of the antibody response to IAV infection or vaccination, was then used to study the effects of repeated annual influenza vaccination and was able to account for the variability seen in the efficacy of repeated annual vaccination across flu seasons. The model inspired others, e.g., Smith et al. (2001) and Wu et al. (2005), who examined strategies for selecting IAV strains to include in a vaccine and suggested methods to use to determine when to update the vaccine.

Within-host evolution models of influenza have also been developed. Perelson et al. (2012) developed a model to examine the probability of drug resistance arising during the course of infection, Russell et al. (2012) examined the possibility of H5N1 evolving to transmit between mammalian hosts, and Luo et al. (2012) developed a model of antigenic immune escape.

Within-host models of viral evolution and immune pressure have also been coupled to epidemiological models. The evolution of the virus can be studied by phylogenetics, and, when coupled to epidemiological models, the term phylodynamics has been coined (Grenfell et al., 2004; Holmes and Grenfell, 2009). Much work has explored the interface between viral evolution, immune pressure, and viral transmission and spread (cf. Andreasen et al., 1997; Gog and Grenfell, 2002; Gog et al., 2003; Girvan et al., 2002; Ferguson et al., 2003; Koelle et al., 2006, 2009, 2010; Boni et al., 2006; van Nimwegen, 2006; Luo et al., 2012).

More complex immune response models with large numbers of equations have been studied using numerical simulations (Bocharov and Romanyukha, 1994; Hancioglu et al., 2007; Lee et al., 2009; Miao et al., 2010a). Because these models include important biological processes omitted in simpler models, they can yield more biologically relevant descriptions of IAV infections. However, because of the large number of parameters these models require, they cannot be fit uniquely to the limited data that exists. Thus, while such models can suggest interesting insights into IAV disease processes, they cannot be used to predict specific disease features quantitatively.

3.4. Toward future multiscale models

If the goal of multiscale IAV modeling is to make predictions about the spread of disease within a population, then the most important feature of a within-host model will be its ability to describe viral kinetic data accurately. Viral kinetic data will be used to link events within the host to disease transmission to a different, susceptible host. In this case, the simplest

possible model might suffice. If an additional goal is to predict the evolution of disease within the host – e.g., disease severity, spatial features, or strain evolution – then it may be beneficial to attempt to incorporate more relevant biology into a model. The goals of the multiscale model should drive the generation of the most appropriate within-host model.

With the advent of more financially accessible high performance computing capabilities, such as on graphical processing units (GPUs), it would be advantageous to attempt to develop more realistic, three dimensional, spatiotemporal models of IAV infection at the tissue scale. Such models could incorporate many more known biological features of IAV infection than can be incorporated into current models. Models could be developed that include multiple cell types, cilia, mucus, trafficking of cells of the immune system, one or more cytokines, and individual virus particles. This is now within reach computationally.

4. Population scale epidemiological models

4.1. SIR models

The standard model for describing infectious disease dynamics at the population scale is the susceptible-infectious-recovered (SIR) model (Anderson and May, 2008). The SIR model, illustrated in Fig. 4, categorizes individuals in terms of their disease status. Ordinary differential equations (ODEs) of the form

$$\frac{d}{dt}S(t) = -\frac{\beta}{N}S(t)I(t) \quad \frac{d}{dt}I(t) = \frac{\beta}{N}S(t)I(t) - \gamma I(t) \quad \frac{d}{dt}R(t) = \gamma I(t) \quad (2)$$

are used to describe the dynamics of an epidemic in terms of the rates of change of disease status. Here, N is the (assumed fixed) total population, β is the transmissibility (encoding both contact structure and probability of infection), and γ is the recovery rate. The SIR model enjoys wide use as the basic building block of population-scale descriptions of numerous diseases. Many aspects of influenza epidemics can be modeled within the SIR framework, and several conclusions immediately follow. First, the growth of the epidemic at early times can be written as (Hethcote, 2000)

$$I(t) \approx I(0)e^{(\beta S(0)/N - \gamma)t} \quad (3)$$

$$I(t) = I(0)e^{(R_0 - 1)\gamma t}, \quad (4)$$

where $R_0 = \beta S(0)/(\gamma N)$. The final size of the epidemic, defined here as the ratio of the final to the initial number of susceptibles, is

$$\frac{S(\infty)}{S(0)} = \exp\left(-R_0\left[1 - \frac{S(\infty)}{S(0)} + \frac{I(0)}{S(0)}\right]\right). \quad (5)$$

The parameter R_0 , known as the “basic reproductive number”, thus characterizes the initial growth of the epidemic and the fraction of susceptibles that become ill, and it represents a very common and simple way to capture the dynamics of an epidemic.

For influenza, the detailed time course of viral shedding suggests that an extension of the basic SIR model is needed (Carrat et al., 2008). Volunteer challenge studies reveal that viral shedding increases rapidly during the first day post-inoculation and peaks *before* symptoms peak, which tends to occur on the second day; detailed time courses are strain dependent. This time course of viral shedding can be accounted for by adding another compartment to an SIR model for asymptomatic but infectious people with population size $I_a(t)$, in addition

to the symptomatic and infectious population $I_s(t)$. Making the reasonable assumption that the infectivities of asymptomatic and symptomatic individuals are different on average, one arrives at the modified SIR model:

$$\frac{d}{dt}S(t) = -\frac{1}{N}S(t)[\beta_a I_a(t) + \beta_s I_s(t)] \quad \frac{d}{dt}I_a(t) = \frac{1}{N}S(t)[\beta_a I_a(t) + \beta_s I_s(t)] - \rho I_a(t) \quad \frac{d}{dt}I_s(t) = \rho I_a(t) - \gamma I_s(t) \quad \frac{d}{dt}R(t) = \gamma I_s(t). \quad (6)$$

This model is quite similar to the SEIR model, which includes an exposed (E) compartment; here, the corresponding compartment is instead labeled as “asymptomatic”, denoting a different epidemiologically relevant population that is infected and infectious. This distinction can be important when modeling scenarios in which knowledge of who is infected is required, such as scenarios involving drug treatment, contact tracing, and surveillance. One can extend this line of thinking to obtain more realistic time courses of infection. For example, one could introduce an exposed or “latent” (L) class, populated directly from the S compartment, which would describe an infected portion of the population that is non-infectious. From the L compartment, both the I_a and I_s compartments would be populated. Such extensions give a more realistic timing of epidemics in part because infectiousness does not peak immediately after infection; this is discussed in more detail below in the context of integral equation formulations.

An important application of the SIR framework is the study of antiviral use and the subsequent emergence of drug resistance. For example, Stilianakis et al. (1998) developed a generalized SIR model with several important features. Because antivirals can be used either as a prophylaxis or as treatment of an illness, the basic SIR model was extended to include those who have received a drug. Asymptomatic classes are important here, as that population infects new susceptibles, but do not receive treatment until they develop clinical symptoms. As the pandemic occurs due to a wild type influenza virus, a resistant strain is allowed to emerge, which requires a complementary set of classes (e.g., asymptomatic, symptomatic, treated/untreated, etc.). This example illustrates the flexibility in the basic SIR framework for treating more complex scenarios. Such deterministic drug resistance models have been extended to stochastic models by Xu et al. (2007). Moreover, this model has been used to examine the roles of *de novo* resistance and transmission fitness for oseltamivir and amantadine under treatment and prophylaxis, with the conclusion that more effort is needed in the areas of quantifying transmission fitness and more advanced computational models that employ realistic human population structure (Regoes and Bonhoeffer, 2006).

Although the SIR model has proven quite useful for describing influenza outbreaks, many details of the disease progression are missed. Influenza affects people of different ages differently (Carrat et al., 2008; Lemaitre and Carrat, 2010), and additional compartments (Keeling and Rohani, 2008) must be added to the basic SIR model to account for such variations. For a single season, in which people age very little, a few compartments (e.g., young, adult, and old) are sufficient. In models intended to be used to study multiple seasons (e.g., evolution studies), it becomes necessary to include migration from one age class to another as each person ages (Hethcote, 2000; Keeling and Rohani, 2008). Age structure can be treated as a continuous variable via partial differential equations; however, despite the fact that age is continuous, discrete class models may correspond more directly to the manner in which data is presented or collected. In age structured models, transmission rates between age classes are accounted for; for example, a transmissibility $\beta(a, a')$ describes the infectivity between two people in age classes a and a' . Similarly, death and recovery rates are also age dependent.

In addition to age structure, the infectivity and recovery rates have a complex time dependence and are not necessarily exponentially distributed. The former can be handled

approximately with an SEIR-type model (Wearing et al., 2005), similar to (6), which is more consistent with known viral titers (Murphy et al., 1973; Douglas, 1975; Richman et al., 1976; Carrat et al., 2008; Canini and Carrat, 2011) and transmission data (Tellier, 2006). Rvachev and Longini (1985) introduced an SEIR-type model that includes time-since-infection information that more accurately accounts for disease progression. In their model, age is discretized into bins, and populations are advanced through the bins; this is essentially a discretization of a McKendrick-von Foerster type PDE model that will be discussed in Section 5.2. Nonexponential distributions can be addressed by employing multiple E and I compartments to shape the distributions (Keeling and Rohani, 2008); in the limit of a large number of compartments, the distributions tend toward a gamma distribution (Lloyd, 2001). Integral equation formulations can be employed (Hethcote and Tudor, 1980; Keeling and Grenfell, 1998) to allow for arbitrarily shaped infectivity and recovery probability distributions. Accounting for such distributions is critical for evaluating the effectiveness of clinical interventions for which the timing at the individual level is essential (Ferguson et al., 2005, 2006; Ciofi degli Atti et al., 2008). The use of such distributions can be illustrated using the model (6). Consider, for simplicity, just the $I_a(t)$ compartment and suppose that we wish to vary the distribution associated with becoming symptomatic. We begin by replacing the differential equation for $I_a(t)$ by an integral equation of the form

$$I_a(t) = I_a(0)e^{-\rho t} + \int_0^t dt' e^{-\rho(t-t')} \cdot \left[\frac{1}{N} S(t') [\beta_a I_a(t') + \beta_s I_s(t')] \right] \quad (7)$$

Note that this predicts that the initial population of asymptomatics, $I_a(0)$, declines exponentially from $t=0$, suggesting that becoming symptomatic is more likely to occur immediately rather than close to its mean value. Now, we can replace (7) by the form

$$I_a(t) = I_a(0) P_a(t) \int_0^t dt' P_a(t-t') \cdot \left[\frac{1}{N} S(t') [\beta_a I_a(t') + \beta_s I_s(t')] \right] \quad (8)$$

where $P_a(t)$ is a probability distribution with the same mean value, but not necessarily the same *shape* as an exponential. Feng et al. (2007) have examined the role of nonexponential distributions on interventions and have concluded that important differences occur when comparing different strategies. Nonexponential distributions have also been employed in the context of a metapopulation model (see Section 4.4 below) by Vergu et al. (2010) where it was shown that, while some aspects of the model are largely unchanged, some details can be significantly different. Although each of these refinements surely adds reality to the simpler SIR models, experimental data of sufficient detail is still lacking to allow for a detailed determination of the inputs to these models; in contrast to determining a numerical parameter, probability functions must be determined.

The SIR models discussed above have been described in terms of their implementation using ODEs. This approach, while quite useful for a wide range of applications, does not capture several realistic features that are important for certain studies. Perhaps most importantly, ODEs describe continuous population variables, whereas real populations are composed of integer numbers of people. Treating a population as an integer number of individuals leads to qualitatively different behaviors when the populations are small. For example, stochastic extinction, a situation in which an epidemic ceases completely when the number of infectives reaches identically zero, can occur when the number of infectious people is small, unless there is another mechanism in the model to regenerate new individuals in the infected class (e.g., for example, through coupling to another geographical region). Stochastic extinction occurs near both the beginning and end of an outbreak or continuously when an infected class never becomes large, which can occur when a class represents people infected by a mutated viral strain, for example. In these cases, the population variables cannot have

real values: because ODEs yield continuous solutions, alternate numerical methods are needed to account for this discreteness. A related issue is the discreteness of the time variable. Although time is continuous in reality, discrete time models can be useful for connecting with the discrete intervals associated with data collection. For all of these reasons, models can be formulated that are either continuous in the state variables and time (e.g., the ODE formulation) continuous in the state variables but discrete in time (update the equation at fixed intervals), discrete in both state variables and time, or discrete in state variables and continuous in time, (Daley and Gani, 1999). The latter two categories require the employment of stochastic approaches, which involve the use of discrete random number generators. Stochastic approaches add additional information about fluctuations around the mean value given by deterministic methods. (The connections between stochastic and mean-field approaches are reviewed elsewhere, in Gillespie, 2007.)

4.2. Individual-based and agent-based models

The term Individual Based Model (IBM) can be applied to models with *demographic* stochasticity, since they treat populations as composed of an integer number of individuals, which allows for heterogeneity among individuals. Compared with compartmental models, each person is his or her own compartment; individuals are not lumped into classes that do not allow for further distinction among those individuals. That is, IBMs track the state of each individual, rather than the population of the different classes. Viewed this way, individuals can be in different spatial locations, have very different times since infection, different numbers of contacts, varying incidents of viral challenges, and so forth (Eubank et al., 2004). Of course, IBMs are far more computationally expensive than many other models, although they are potentially far more accurate if the relevant details (e.g., sociological, disease progression, etc.) are known.

None of the models discussed so far explicitly include social behavior. Models that do are termed agent-based models (ABMs). ABMs originate in the sociological sciences, where they are used to describe the evolution of events that are determined by social actors (e.g., individuals, corporations, or groups) that have traits such as beliefs, goals, habits, and the ability to learn (Gilbert, 2007); broadly speaking, ABMs form the discipline of computational sociology. The term ABM has become blurred as it gains wide use across many disciplines. ABMs are often considered synonymous with IBMs; in fact, the term ABM is also used for models of any kind in which a discrete entity is employed at any scale, including populations, geographic districts, governments, and viruses themselves. The term ABM, however, appears to have first been used in the economics literature and is still used today in the sociology literature to be an IBM in which the agents are a “collection of autonomous decision-making entities” (Bonabeau, 2002; Epstein, 2009). The term ABM has taken on such a broad meaning that it is now often used to describe models used in fields as disparate as political science, economics (Axelrod, 1997), epidemiology, ecology (Grimm et al., 2006), and infectious disease biology (Bauer et al., 2009). The ABM-IBM distinction is rarely used in the influenza literature and the terms IBM and ABM tend to be used interchangeably. In fact, IBMs and ABMs in this context tend to fall short of including decisions or beliefs but do include social contact networks, social responses in a pandemic (e.g., quarantine, contact tracing, fear, errors, etc.), detailed population structure, census and land-use information, population mobility, contact types (e.g., nurse, child in school, etc.), and so forth. Behavioral changes due to, for example, the quality of healthcare and the quality of information during an epidemic (Kitchovitch and Lio, 2011) have been explored with an SIR model on a social contact network (see next section).

Ferguson et al. (2005) have developed an IBM to examine strategies for containing influenza pandemics that arise in southeast Asia. In their model, detailed population data was used to simulate an epidemic among the 85 million residents of Thailand and parts of its

neighboring countries. Household, school and workplace population compartments were included in the model, with different disease transmission rates for each of these compartments, and individuals moved between these compartments throughout each day. This model was subsequently used to understand the impacts of mitigation strategies in pandemics, including the use of antivirals, vaccines, and social distancing measures (Ferguson et al., 2006). When the spatial scale exceeds the country scale, IBMs become very computationally expensive, and other methods might be more efficient. Below, the metapopulation model, in which spatial regions are treated with less detail, will be introduced; recently, an explicit comparison between an IBM and a metapopulation model has been carried out (Ajelli et al., 2010) for influenza-like illnesses in Italy. However, as computational resources have improved, entire continents have been modeled; Merler et al. (2011) and Merler and Ajelli (2010) have modeled over 500,000,000 people in IBM studies of Europe.

Germann et al. (2006) have simulated a pandemic in the United States using a detailed IBM. Their model includes 281 million individuals distributed into 2000 person communities. The individuals have contacts associated with their mixing groups, which include work, school, preschool, play groups, household, household clusters, neighborhoods, and communities. Contacts change during each two 12-h day-night cycle (see Section 4.3). Transportation was included using worker flow data available from the U.S. Census. Social behavior was modeled through assumptions about voluntary social distancing during a pandemic. This highly detailed model was used to explore a wide range of intervention strategies using targeted antiviral prophylaxis, imposed social distancing, vaccination, and travel restrictions. A closely related model was used to examine the effectiveness of vaccination strategies during the 2009 H1N1 pandemic by Yang et al. (2009). Because of the details included in the IBM, the authors were able to address specific issues associated with spread through different age groups (school age in particular) and households.

4.3. Networks

The simple homogeneously mixed SIR-type models that we have considered above ignore all details of each interpersonal interaction. However, in reality, each individual cannot, in general, be thought of as an average spreader of disease, since the distribution of contacts that we make is highly dependent on our personalities, our jobs, what time of year it is, how we socialize, and many other factors. At a larger spatial scale, people cluster into groups. Groups can be friends at school, coworkers in the same building, members of a community, people who carpool, and so forth. Including the details of contact structure and clustering is most obviously done in the context of an IBM in which each person has a specific number of contacts, perhaps drawn from a known distribution.

A formal method for describing epidemics between individuals connected by contact information is with a graph or “network” (Watts, 2004; May, 2006). A graph $G(V;E)$ is a set of points or “vertices” V that represent individuals, and connections or “edges” E that represent contacts. Each vertex/person can have a different number of edges; just how many edges a vertex has is referred to as the degree of that vertex. In general, a given network will have a distribution of degrees. An example of such a graph for ten people is shown in Fig. 5. In this simple graph we see a network of ten people, four of which are susceptible, three of which are infectious, and three who are recovered. Each person is connected to his or her two neighbors, representing close spatial ties such as sharing an office, and to more distant individuals via edges that span the network, representing contacts outside the local environment such as is experienced by members of a carpool. Using the usual rules of SIR models, the epidemic can be evolved on this graph, and we can learn how the contact structure (Read and Keeling, 2003) serves to modify the dynamics of the epidemic. For

example, how important are the edges that extend across the graph, and the loops that they form, for disease dynamics?

Much of what we know about disease propagation on networks stems from mathematical and statistical analyses of network properties (Newman, 2010). Often, the network is constructed using lattice configurations (Keeling and Rohani, 2008) and/or simple statistical rules (Newman, 2002B). More detailed networks can be constructed that incorporate clustering (neighbors of a vertex tend to be neighbors of each other) and assortativity (high degree vertices tend to attach to other high degree vertices) (Badham and Stoker, 2010; Newman, 2002a). These methods serve to qualitatively capture the role that networks play in the spread of diseases like influenza, but are not calibrated using social data, which suggests very specific network structures (Wasserman and Faust, 2009; Hamill and Gilbert, 2008). As mentioned above, Kitchovitch and Lio (2011) have examined how social behavior is modified on a network; in their work, networks were constructed with strong community clustering with varying awareness of the disease. Incorporating such human behavior is likely to be important for developing realistic forecasting models; for example, such information could be used to optimize contact tracing (Eames et al., 2010; Fraser et al., 2004). Network structure has been employed to examine influenza vaccination strategies as well (Bansal et al., 2006).

Experimental network science is an important and exciting developing area for understanding influenza outbreaks at the population scale; because of the difficulties in predicting the relevant network structure for influenza, it is imperative that we measure it empirically. Christakis and Fowler (2010) constructed a social network at an American college using an interview process that focuses on friendship networks. In their strategy, individuals were selected at random and asked to identify their friends. From this information, a network was constructed in which central individuals were identified. Data taken for the 2009–2010 influenza season for the participants in the study supports the notion that epidemics spread first through the central individuals with a two day lead. This interesting result is based on the friendship paradox property of social networks: friends of randomly selected people are more linked and central than the original random set, on average. Salathe et al. (2010) have used wireless sensor technology to measure close proximity interactions at an American high school on a typical day. The wireless sensors are referred to as motes (see Fig. 6), which use radio frequency signals to communicate with other motes. These measurements, and a computer simulation of an outbreak that is consistent with them, suggest optimal strategies for immunization. Because influenza is usually spread by droplets, such proximity data is central to understanding the forms of actual transmission networks. More standard methods (Wasserman and Faust, 2009) such as surveys (Mossong et al., 2008) have also been used to reveal details of realistic network properties relevant to the propagation of respiratory diseases.

Because simulations of disease dynamics on a network are computationally intensive, approximate models are indispensable. The state of a network can be described by a joint probability distribution function for the states of each of the, say, N vertices (reflecting the disease states of the individuals). This function contains more information than we typically require and is cumbersome for actual calculations. Using methods borrowed from statistical sciences, it is possible to consider reduced distribution functions for fewer variables $M < N$; these reduced functions couple to more complex functions through a hierarchy that must be truncated. Most often a pair approximation (Rand, 1999) is made in which correlations between pairs are retained. In Fig. 5, for example, the evolution equations for the “bonds” (SS, II, RR, SI, SR, IR) can be obtained, but with the neglect or approximation of higher-order information encoded in three-vertex correlations (e.g., SSI, SRI, etc.). Although this method neglects a considerable amount of information that is contained in the original

graph, the computations are substantially easier (Keeling, 1999) and, quite often, very accurate (Newman, 2010).

Beyond describing the general features of epidemics more accurately, an important goal of including network structure is to suggest potentially more efficient intervention strategies. In particular, targeted vaccination and antiviral prophylaxis strategies can be suggested that are predicted to more efficiently slow or extinguish a pandemic, thereby reducing morbidity and mortality. Hartvigsen et al. (2007) investigated five different vaccination strategies on circulant graphs ranging from random to small-world to regular within an SIR model of a population of 10,000. They found that vaccinating vertices with the largest degree resulted in the most effective strategy for all cases. However, targeting individuals with the largest clustering coefficient, relative to random, resulted in more infections.

4.4. Metapopulation models

Network models, while capable of incorporating spatial information, become cumbersome when the level of clustering becomes very high. This situation stems from the facts that direct simulations using networks have poor scaling for large populations, and that models built around pair approximations are no longer accurate. For example, consider two interacting clusters that represent a small town in Australia and a small town in Canada; clearly, it makes sense to treat the small towns as homogeneously mixed populations with a weak connection. Such models, in which a set of homogeneously mixed populations is connected, are referred to as metapopulation models because they are populations of subpopulations. Subpopulations are referred to as “patches,” and their disease dynamics can have various levels of synchronization with other patches. Metapopulation models are useful when the spatial scales of interest begin to exceed what network models can reliably handle.

Various methods have been developed to model metapopulations. In the most detailed versions, populations are labeled in terms of their places of origin i and where they reside at the moment j ; for example, $S_{ij}(t)$ is the population of *susceptibles* from patch i that happen to be in patch j at the moment. These models require explicit human movement, at “travel” rate τ , and “returning” rate ρ . Again, subscripts are used to denote which patches the travel is between. Neglecting birth and death processes, the SIR model extended to a metapopulation is

$$\begin{aligned}
 \frac{dS_{ii}(t)}{dt} &= -\beta_{ij}S_{ii}(t)\frac{I_{ii}(t)+I_{ji}(t)}{N_{ii}(t)+N_{ji}(t)} - \tau_{ij}S_{ii}(t) + \rho_{ij}S_{ij}(t) \\
 \frac{dS_{ij}(t)}{dt} &= -\beta_{ij}S_{ij}(t)\frac{I_{jj}(t)+I_{ij}(t)}{N_{jj}(t)+N_{ij}(t)} + \tau_{ij}S_{ii}(t) - \rho_{ij}S_{ij}(t) \\
 \frac{dI_{ii}(t)}{dt} &= -\beta_{ij}S_{ii}(t)\frac{I_{ii}(t)+I_{ji}(t)}{N_{ii}(t)+N_{ji}(t)} - \tau_{ij}I_{ii}(t) + \rho_{ij}I_{ij}(t) - \gamma_{ij}I_{ii}(t) \\
 \frac{dI_{ij}(t)}{dt} &= \beta_{ij}S_{ij}(t)\frac{I_{jj}(t)+I_{ij}(t)}{N_{jj}(t)+N_{ij}(t)} + \tau_{ij}I_{ii}(t) - \rho_{ij}I_{ij}(t) - \gamma_{ij}I_{ij}(t) \\
 \frac{dN_{ii}(t)}{dt} &= -\tau_{ii}N_{ii}(t) + \rho_{ij}N_{ij}(t) \\
 \frac{dN_{ij}(t)}{dt} &= \tau_{ii}N_{ii}(t) - \rho_{ij}N_{ij}(t)
 \end{aligned} \tag{9}$$

Note that additional variables $N_{ij}(t)$ are needed to keep track of the total number of people in each patch. For a number of patches N_p , mechanistic models of this type have a poor computational scaling of $\mathcal{O}(N_p^2)$, making them difficult to use in practice. In fact, it is not always necessary to employ such a detailed model when there is a clear separation of time

scales. Commuter travel occurs on a scale of hours, whereas influenza disease progression occurs on the scale of a week or longer. Using this information, it is possible to construct a gravity model that scales as $\mathcal{O}(N_p)$ (Keeling and Rohani, 2002).

Because the connections between patches can be weak, metapopulation models are highly susceptible to stochastic effects (Lande et al., 1998). Repeated stochastic extinctions can occur between two weakly coupled patches, unless and until a sufficient level of infectious invasion has occurred.

Novel methods are under development for tracking the timing and spatial spread of influenza. For example, search terms on Google turn out to be reasonable indicators of global influenza activity (Ginsberg et al., 2009) as compared with Centers for Disease Control and Prevention data, and Google has developed the Flu Trends site www.google.org/flutrends for this purpose. See Fig. 8 for a comparison of Google Flu Trends activity in the United States and Australia, and Fig. 9 for a snapshot of Google Flu Trends activity around the world. Similarly, Signorini et al. (2011) have shown that Twitter also represents a source of useful keywords that indicate influenza outbreak patterns. To incorporate the latter two forms of information requires generalizations of the SIR-like models discussed so far to include spatial information.

4.5. Human mobility patterns

The key ingredient to span larger geographical regions is information about human movement patterns. These patterns are traditionally described in terms of five features: mode of travel, number of trips, route, and origin and destination (Kitamura et al., 2000). For influenza models, the mode of travel is most often divided into the two classes of commuter and airline traffic. Routes for airline traffic are taken from known flight schedules, as are the destinations and origins. Commuter traffic is connected with local population patterns and census data. Balcan et al. (2009) have analyzed data from 29 countries and have shown that commuter flows exceed airline flows by an order of magnitude; however, *large scale* epidemic patterns are almost entirely determined by airline traffic. Nonetheless, commuter traffic cannot be neglected entirely, as it leads to local synchronization. General properties of epidemics across a range of spatial scales have been investigated by Watts et al. (2005). In their model, a nested hierarchy of successively larger domains is used to connect domains in which the assumption of homogeneous mixing is expected to apply. They find the interesting result that the basic reproductive number R_0 is not a good indicator of the final epidemic size because of the sensitivity of an epidemic to the structure of the population; that is, for fixed R_0 , the duration and final size of the epidemic depend strongly on the transport at different scales, in contrast to the simple homogeneous result of (5).

A useful concept for describing human mobility across varying length scales is the transportation gravity model. These models characterize commuting flows as proportional to products of powers of the populations of the two regions and inversely proportional to some measure of the distance d_{ij} between the regions. Fig. 7 shows an example of how a region might be divided for such a model. For example, Balcan et al. suggest the form

$$w_{ij} = \mathcal{C} \frac{N_i^\alpha N_j^\gamma}{\exp(d_{ij}/r)}, \quad (10)$$

where α , γ , and r are the parameters that commuter data is fit to. It is important to keep in mind that the values of the parameters depend upon the resolution of the metapopulation model used to fit the transportation data.

A metapopulation gravity model was used by Viboud et al. (2006) to examine synchrony in the United States. Based on the analysis of data between 1972 and 2002, spatial waves in the spread of influenza were observed to occur in hierarchies. This country-scale epidemic behavior was modeled using detailed travel patterns. A major result of this work is that, while all forms of human movement are important, the strongest correlation between disease movement and human movement is for workflows. In their model, workflows were modeled by a form similar to (10), which in their notation reads

$$C_{ij} = \theta \frac{P_i^{\tau_1} P_j^{\tau_2}}{d_{ij}^p}. \quad (11)$$

Here C_{ij} is the community workflow between population P_i and P_j of community i and j , respectively, and the remaining constants are tuned to workflow data. Intriguingly, the authors found that the value of p was itself distance dependent, being larger (> 3) for movement up to 119 km and nearly zero (> 0) for larger distances. This result illustrates a limitation of the simple forms in (10) and (11). The scaling with respect to population sizes indicates that smaller populations are more important per capita, since both τ 's were less than unity. From this model the authors were able to connect patterns of workflows to spatial correlations of influenza in the U.S.

Despite the wide use and success of the gravity model, it has several undesirable properties. The model contains at least three, and often many more, poorly controlled parameters. Furthermore, in the case in which the populations of the destination and origin are very different, the model can predict that the smaller population is completely depleted into the larger city or that the smaller population is inundated with flow from the larger population. Merler and Ajelli (2010) have constructed an improved gravity model that is dependent on the gross domestic product per capita. Recently, Simini et al. (2012) have addressed six major limitations with the gravity model and have suggested a new model based on employment opportunities that they refer to as a “radiation model” which improves upon previous gravity models.

Airline data, on the other hand, can be described more accurately and in greater detail. An important early work on the global spread of influenza is that of Rvachev and Longini (1985). In their work, 52 of the world's largest cities, including cities on all continents, were incorporated into a model for forecasting the spread of a single virus strain during a pandemic. Epstein et al. (2007) have refined this model to explore the control of pandemic flu by including mobilities among 155 major cities; their airline data was obtained from several sources, including the U.S. Census Bureau and the United Nations Department of Economic and Social Affairs. One expects that the refined model of Epstein et al. is an improvement over the earlier work of Rvachev and Longini because of the use of modern travel patterns between more cities. Colizza et al. (2007) examined containment interventions using 3100 cities in 220 different countries for nearly complete coverage of the globe. In contrast to models that introduce additional parameters, such improvements incorporate data that is easily measured and is independent of disease parameters. An interesting application of the use of global flight patterns is the investigation of disease spread due to mass gatherings, such as sports or religious events, by the Bio. Diaspora Project (Khan et al., 2009). However, there are no extant convergence studies to our knowledge.

4.6. Putting it all together

Although many important questions about influenza can be addressed adequately using modest SIR-type models, some important questions are addressed only with large-scale

models. For example, the global circulation and evolution of influenza (Russell et al., 2008) and pandemic preparedness (Ferguson et al., 2006) require sufficiently complex models. As we have discussed above, the models of Rvachev and Longini (1985), Germann et al. (2006), and Epstein et al. (2007), among many others, have continued to add refinements to all aspects of influenza modeling, including human movement patterns, disease progression, contact/network structure, age structure, and demographic details. This advance has been made possible in part by more detailed knowledge of the inputs to these models, but also through advances in high performance computing. One computationally intensive tool for studying epidemics is EpiSims (Eubank et al., 2004), which encodes an agent based model that incorporates census data, land-use data, age structures, income data, and human mobility data. Moreover, this model incorporates a dynamic network to describe contacts; this extremely large and detailed social contact network was built upon TRANSIMS, a simulation tool developed for understanding transportation infrastructure. The EpiSims model is also multiscale in the sense that within-host disease progression and between-host transmission are both included. The EpiSims tool has been used to examine mitigation strategies for a smallpox outbreak, among other applications. A comparison of several detailed epidemic simulation codes was carried out in a study of targeted layer containment of an influenza pandemic (Halloran et al., 2008).

5. Multiscale models

5.1. Intrahost spatial models

Intrahost multiscale models can be categorized into two distinct types depending on whether they incorporate intracellular details or spatial information. Few models have been constructed that couple intracellular and extracellular responses for influenza. A generic model has been developed (Haseltine et al., 2005), not specific to influenza, that incorporates multiple intracellular components and various cell types. Models of this type could be applied to pharmacokinetic studies of influenza infections, for example. Today, the paucity of experimental data precludes us from quantifying the value of these more complex models. Haseltine et al. addressed this ubiquitous situation by generating synthetic experimental data using their model and fitting simpler models to that “data”. In some cases these fits are rather poor, which reveals the conditions that the multiscale model would need, which in turn can motivate specific experiments. Later, they further examine their model to understand the conditions under which the intracellular and extracellular portions decouple (Haseltine et al., 2008). Such models merit more careful attention and should be constructed within the context of influenza to reveal details of the coupling that are likely disease dependent.

Few spatial models have been developed at the extracellular level. A cellular automata (CA) model has been developed (Beauchemin et al., 2005) in which a two dimensional lattice is used to describe inhomogeneities in space. This model has been used to examine the assumption of homogeneous mixing (Beauchemin, 2006) with the conclusion that the dynamics of infection is greatly impacted by spatial infection distributions. CA models employ transition rules between lattice sites at each time step. It is important to relate these rules to empirically observed viral transport (Anekal et al., 2009). Most often, a continuum deterministic model is employed in which diffusion is described by

$$\frac{\partial V(\mathbf{r}, t)}{\partial t} = D_V \nabla^2 V(\mathbf{r}, t), \quad (12)$$

where D_V is the virion diffusion coefficient. The diffusion coefficient, which is related to the mean squared displacement, is an experimentally measurable quantity; rules in CA models must be tuned to correspond to the observed diffusion coefficient. Unfortunately, at the

present time, little is known about the diffusion coefficient of influenza virions in tissue environments, and estimates must be made using the Stokes–Einstein relation (Beauchemin et al., 2006). It is worth noting that these authors were unable to reproduce their experimental data using the Stokes–Einstein value, and concluded that the actual value is closer to 10^3 times this value based on a measure of patchiness in both the experiments and the simulations. More sophisticated mathematical formulations have been developed that incorporate stochastic reactions and transport (Ferm et al., 2010); such formulations could usefully be applied to influenza infection dynamics.

A caveat must be made, however, regarding models of diffusion described by Eq. (12). Mucus is a highly complex environment (Lai et al., 2010) in which the movement of small objects is not described by Eq. (12), which implicitly assumes that the mean-squared displacement is linear in time for long times. Transport that does not have such a form is referred to as anomalous diffusion. Stochastic models that include obstacles and binding (Saxton, 1994, 1996) might prove useful for describing influenza transport; however, single influenza virus particle tracking experiments are needed to inform definitive models.

5.2. Connecting viral load to transmission

It is possible to couple in-host and between host models with the use of an age structured model in which age refers to the “infection age” of an ill person. For an SIR system this can be written as

$$\frac{dS(t)}{dt} = -S(t) \int_0^T d\tau \beta(\tau) I(\tau, t) \quad \frac{\partial I(\tau, t)}{\partial t} = -\frac{\partial I(\tau, t)}{\partial \tau} - \gamma(\tau) I(\tau, t) \quad I(0, t) = S(t) \int_0^T d\tau \beta(\tau) I(\tau, t) \quad \frac{dR(t)}{dt} = I(T, t). \quad (13)$$

The variable τ is the time since infection, and the infectivity $\beta(\tau)$ and recovery $\gamma(\tau)$ are now explicitly time dependent. The connection to in-host dynamics can be made in a variety of ways, with the simplest being

$$\beta(\tau) = aV(\tau) \quad \gamma(\tau) = gF(\tau), \quad (14)$$

where it is assumed that the infectivity is proportional to the viral load, $V(\tau)$, and the recovery is proportional to the level of immune response, $F(\tau)$. The system of Eqs. (13) is variously referred to as the McKendrick–von Foerster (MvF) or Lotka–McKendrick system (Arino et al., 1998; Castillo-Chavez et al., 1989). It is worth noting that a great deal of work utilizing models of the form (13) have been used in studies of viral evolutionary dynamics (Coombs et al., 2007; Amaku et al., 2010; Luciani and Alizon, 2009; Mideo et al., 2008; Day et al., 2011; Lange and Ferguson, 2009), where these linked models are referred to as “nested” models (Mideo et al., 2008).

At the level of complexity associated with the forms given in (14), we are assuming that the viral load and immune response are *given*. Models of this form, in which there is no feedback to lower scales from higher scales, are sometimes referred to as “inessential” (Mideo et al., 2008). In Fig. 10 we show how information could flow from different scales; inessential models do not contain the dashed line, which is necessary for evolutionary studies that examine evolution at different scales. The advantage of inessential models is that experimentally measured forms for $V(\tau)$ and $F(\tau)$ can be incorporated. The model is also useful in the case in which these forms arise from the solution of a within-host model; in this case, the within-host model only needs to be solved once. Models of this kind have been used extensively to study co-evolution in host-parasite systems, with little application to influenza epidemiology to date. Such a model was used in the seminal work of Rvachev and Longini (1985), however, in a discretized version in which both time variables (τ , t) appear in one-day intervals. Their model, one of the first to describe the global spread of

influenza, is discussed in more detail above in that context. Epstein et al. (2007) employed the Rvachev and Longini model in their studies of travel restrictions. Because there is no feedback from the larger scale back to the smaller scale, such models are only multiscale in the sense that they incorporate a more refined treatment at the finer scale, either through experimental data or as a result of numerical results. Still missing from (14) is the determination of the parameters a and g . The parameter a can be thought of as a mean infectivity modulated by the viral load profile to account for different levels of transmission during the illness. Similarly, the parameter g arises from the fact that recovery is less likely early in the illness and becomes more likely later when the required response of the immune system has been achieved. Today, very little is known about the values of a and g , or their generalizations in models more complex than (14); further careful studies of the transmission process and the immune response are needed.

In Section 3 we described within-host models that yield viral load dynamics, which need to be connected with viral shedding via coughing and sneezing to the between-host infectivity parameter. Hayden et al. (1998) have investigated the role of cytokine response in symptom formation in influenza infections. Handel et al. (2007) have fit nasal discharge versus viral load to a four-parameter Hill function. From this relation, they compute the total (integrated) amount of shedding and assume that it is proportional to R_0 . However, the connection of nasal discharge shedding to actual infection is much less well known. For this reason, connecting within-host models to, for example, network models remains a challenge. Because droplets can be suspended in the air for hours, and because some of the shedding is likely spread through contact with environmental surfaces, it is largely unknown how to estimate the contact structure that would inform the edge topology in network models. This situation for influenza should be contrasted with other diseases that do not have this challenge, such as diseases spread by needle users and/or sexual contact.

6. Conclusions, summary, and outlook

Mathematical modeling of influenza at all time and length scales has yielded useful insights. Here, we have reviewed models at each of the scales from the cellular to the population scales. Multiscale modeling, in which some or all of the scales are linked, has been highlighted as a promising next phase of influenza modeling. Recent advances in this area have been driven by advances in experimental measurements that reveal, on one extreme, spatial inhomogeneities at the cellular level and, at an intermediate scale, measurements of social network patterns at a community scale and, at the other extreme, disease patterns driven by transportation networks.

The increasing availability of experimental data is allowing models to become more complex and accurate, but more experiments are needed to improve models. More experiments are needed to improve our understanding of the transport of influenza virus particles within mucus of the respiratory tract. For example, the diffusion coefficient of influenza virus in a mucus membrane is unknown; nor is it known whether the diffusion of IAV is normal or anomalous. Knowing how IAV diffuses through mucus could be very useful for developing new IAV models because heterogeneous patterns observed at the tissue level suggest that transport at the tissue scale is an important determinant of the initiation of infection, the tissue-scale spread of infection, and viral shedding to susceptible hosts.

There are many outstanding questions that provide ample opportunity for further research in the nascent area of multiscale modeling of IAV infection. The connections between tissue-level spatial patterns of viral load and both disease initiation and transmission remain some of the least understood aspects of the spread of influenza. Because of this lack of knowledge, the connection between within-host and between-host modeling is relatively primitive. As

new experimental data is generated and models are improved, their accurate portrayal of the underlying biology and epidemiology will allow us to understand this disease more fully and better mitigate its consequences.

It would be hoped that with improved modeling capabilities, public health questions can be addressed more optimally. With better models, we can better answer questions of how best to allocate medications and vaccines, to find the right balance between treatment and prophylaxis in a population, to delay or reduce the development of drug resistance, to predict the severity of an epidemic or pandemic, to predict the consequences of poor vaccine match, and to develop economic analyses of influenza epidemics and intervention strategies.

Acknowledgments

We gratefully acknowledge the support of the U.S. Department of Energy through the LANL/LDRD Program and NIH contract HH5N272201000055C.

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HIGHLIGHTS

- Computational and experimental advances are making more detailed models of infection possible.
- To date, influenza infection has primarily been modeled within the host or at population scales.
- Transmission data can be used to link within-host and population scales.
- Multiscale models can be used to study public health interventions and disease biology.

Fig. 1. IAV infection of normal human nasal epithelial cells in air-liquid interface culture. The cultures were infected with either a human (a) or avian (b) IAV strain, fixed 7 h after infection, and immunostained for cilia (gray) and virus antigen (red). Human strains predominantly infect non-ciliated cells, and avian strains predominantly infect ciliated cells. Tissue scale models can be designed that take these target cell differences into account, depending on the host of the strain to be modeled. (Bars = 10 μ m.) (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

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Fig. 2.

Spread of human and avian IAV in air-liquid interface cultures of normal human tracheobronchial epithelial cells, in the course of multicycle infection. Cultures were infected with a human strain (a) or an avian strain (b) at an MOI of 0.02, fixed 24 h after infection, and immunostained [virus (red), cilia (gray)]. Clusters of adjacent cells are infected in both human and avian IAV infections, often with large, open areas of uninfected cells between infected cell clusters. Thus, this data suggests that innate immune responses may not protect adjacent cells from productive infection, and that tissue scale models should hypothesize that interferons induce antiviral states over larger times and distances. (Objectives, $\times 10$. Bars = $25\mu\text{m}$.) (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.) Reprinted from Matrosovich et al. (2004a), © the National Academy of Sciences, 2004.

Fig. 3.
Within-host compartmental model of IAV infection.

Fig. 4. The basic susceptible–infectious–recovered (SIR) compartmental model. Susceptible people become infected by infectious people according to a transmissibility β and permanently recover at rate γ .

Fig. 5.

A simple network of ten people described by an SIR model. This form of network is expected to loosely correspond to realistic contact structures appropriate to disease dynamics. The structure of this network, in that each person (vertex) has a contact (edge) with their neighbors and *possibly* a contact across the network, is referred to as a “small-world network.” Note that the vertices can be in three possible states, S, I, and R, and the edges describe all possible type of contacts: SS, II, RR, SI, SR, and IR.

Fig. 6.

A mote used to measure proximity data for constructing realistic social networks. Motes communicate with each other, and possibly with stationary stations, to provide contact structure data between individuals and relative to chosen landmarks.

Fig. 7.
Metapopulation models: dividing geographical space according to population densities.

Fig. 8.

A comparison of Google Flu Trends data for the United States (blue) and Australia (red). Normalized, aggregated flu search activity for both countries is represented as standard deviations from the baseline for that country, which is set to 0. The baseline for each country is its average flu search activity level, as measured over many seasons. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

Fig. 9.

Google Flu Trends data for the world on March 11, 2013. Regions are shown in colors on a scale ranging from light green (indicating minimal flu activity), through yellow (low flu activity), orange (moderate flu activity), and red-orange (high flu activity), to dark red (intense flu activity). White regions indicate no data. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

Fig. 10.
Coupling within-host models to the population scale.