Streptococcus pneumoniae (the pneumococcus) is the leading cause of community-acquired pneumonia. The incidence of pneumococcal pneumonia is greatest at the extremes of age and in individuals with medical comorbidity. Pneumococcal infection causes approximately 2 million deaths globally and costs hundreds of billions of dollars per annum. Streptococcus pneumoniae frequently colonizes the nasopharynx and from the nasopharynx, pneumococci can spread directly via the airway to the lower respiratory tract, causing pneumonia, or to the sinuses or middle ears, causing medical morbidity. Bacteria may also penetrate the epithelial cell surface, resulting in local infection or bacteremia. The pleura and meninges can be seeded by local spread of infection or by bacteremia. Invasive pneumococcal disease (IPD) is defined as isolation of bacteria from a normally sterile site, such as blood, cerebrospinal fluid, or pleural space, and results from invasion of the respiratory epithelium.

Antimicrobial therapy and vaccination have been central to the clinical approach to pneumococcal disease,
but efforts to enhance the host response, other than through immunization, have received limited attention. This is somewhat surprising because colonization with the pneumococcus is widespread and development of pneumonia is a comparatively rare event. This review focuses on recent findings relating to the microbiology and pathogenesis of pneumococcal pneumonia and includes data from murine models of pneumococcal disease. Murine models of pulmonary infection have provided valuable insights because they have allowed the manipulation of molecules or specific cell populations to test their role in a model system of pneumococcal pneumonia.

**GENETIC VARIATION**

*Streptococcus pneumoniae* demonstrate transformation and horizontal gene transfer, ensuring a high rate of variation in the genome. Pneumococci acquire environmental DNA from a number of sources, including the extracellular matrix of pneumococcal biofilms, which contain DNA, and via microbial fratricide, whereby noncompetent siblings are lysed, facilitating release of chromosomal DNA. The “distributed-genome hypothesis” posits that pathogenic bacteria have a large number of noncore genes (the “bacterial supragenome”) not present in all strains, maintaining diversity. Analysis of eight pneumococcal strains colonizing children, and comparison with nine freely available strains, revealed that 54% of genes were not found in all strains and that any individual strain possessed only 21% to 32% of these noncore genes. Whole genome sequencing of 240 isolates of serotype 23F, strains associated with multidrug antimicrobial resistance, has revealed that 74% of the genome was altered in at least one of the sequenced strains. Recombination hot spots were not just related to genes encoding antimicrobial targets, but were also concentrated in genes encoding capsular biosynthesis and adhesins, targets of the immune response. These findings suggest that current vaccine strategies provide, and future vaccine strategies should provide, a potent selective pressure for pneumococcal genetic evolution and that pneumococci will likely continue to evolve in response to the selective pressure exerted both by the immune response to vaccines and by antimicrobials.

**Capsule Diversity**

At least 92 different serotypes of pneumococcal polysaccharide capsule exist, the most recently described being serotype 11E. A systematic review and meta-analysis compared the risk of death with specific serotypes, using serotype 14 as the reference strain. Results were compared with adults with low comorbidity scores to reduce confounding by host factors. Serotypes associated with an increased risk of death during bacteremic pneumonia included serotypes 3, 6A and B, 9N, and 19F, whereas serotypes 1, 7F, and 8 were associated with a lower risk of death. The serotypes associated with an increased risk of death were those strains that cause lower rates of IPD following pediatric colonization. These strains are less likely to invade but when they do, IPD results in a higher mortality than observed for other strains (Table 1). Colonizing strains behave as “opportunistic infections” in individuals with medical comorbidities, whereas strains with a higher propensity to cause IPD when carried in the nasopharynx behave as “primary pathogens” in medically fit individuals.

The ability of pneumococci to colonize and invade has been related to a variety of factors, including phase variation. In this process, the colony morphology for an individual serotype can vary between transparent and opaque when viewed on transparent agar with oblique transmitted light. Transparent phenotypes colonize the airway readily, whereas opaque phenotypes are well able to survive in the bloodstream and resist phagocytosis. Opaque phenotypes have a number of characteristics favoring survival following tissue invasion, one of which is the presence of increased encapsulation.

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**Table 1—Influence of Capsular Serotype on Clinical Course and Biologic Behavior**

<table>
<thead>
<tr>
<th>Study</th>
<th>Characteristic</th>
<th>Serotypes Associated With Colonization and Impact (Relative to Reference Strain if Applicable)</th>
<th>Serotypes Associated With Increased Rates of IPD Studied and Impact Relative to Reference Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weinberger et al&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Mortality with IPD</td>
<td>↑ with 3, 6A, 6B, 9N, and 19F vs 14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>↓ with serotype 1, 7F and 8 vs 14</td>
</tr>
<tr>
<td>Weinberger et al&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Resistance to nonopsonic killing by neutrophils</td>
<td>↑ with 6A, 6B, 9N, 14, 19F, and 23F vs 9V</td>
<td>↓ with serotype 1, 4, 5 vs 9V</td>
</tr>
<tr>
<td>Sanchez et al&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Accessibility of adhesins</td>
<td>↑ 6A, 23F</td>
<td>↓ 4</td>
</tr>
<tr>
<td>Sanchez et al&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Ingestion by alveolar macrophages</td>
<td>↑ 6A, 23F</td>
<td>↓ 4</td>
</tr>
<tr>
<td>Hyams et al&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Deposition of complement</td>
<td>↑ 6A, 23F</td>
<td>↓ 4, 7</td>
</tr>
<tr>
<td>Weinberger et al&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>Capsule</td>
<td>↑ with 6A, 6B, 19F, and 23F vs 9V</td>
<td>↓ with serotype 1, 4, 5, 7 and 14 vs 9V</td>
</tr>
</tbody>
</table>

Serotypes of *Streptococcus pneumoniae* were grouped as either associated with colonization and relatively low rates of IPD per 100,000 acquisition events or as being associated with relatively high attack rates of IPD per 100,000 colonizing events, as described by Sleeman et al<sup>2</sup> in a pediatric population, and the relative frequency of various characteristics compared. IPD = invasive pneumococcal disease.

<sup>a</sup>The behavior of serotype 14 has some features of strains associated with low and some features associated with high attack rates.
Differences in the capsule among serotypes are also an important determinant of the variation in biologic behavior among serotypes. Colonizing strains are more resistant to nonopsonic killing by neutrophils, a key determinant of nasopharyngeal carriage. Capsular switch studies, in which different serotype capsules are swapped onto an otherwise identical genetic background, confirm that differences in capsule account for much of the variation in biologic behavior. Capsular serotype also influences tissue invasion; serotype 6A or 23F capsule is associated with reduced accessibility of adhesins, including pneumococcal surface protein, choline-binding protein A, and pneumococcal serine-rich repeat protein (PsrP), resulting in decreased adherence to epithelial cells and increased ingestion by alveolar macrophages (AMs), when compared with the more invasive serotype 4 capsule. Capsules from invasive serotypes, such as serotype 4 and 7F, are more resistant to complement deposition and opsonin-dependent phagocytosis than are capsules associated with colonization, such as 6A and 23F, although variation in complement deposition is not mediated solely by capsular serotype.

Thus, colonizing and invasive strains have evolved to exploit unique ecologic niches and have acquired the necessary adaptations to evade the key immune responses controlling bacterial replication in their location (Fig 1). The colonizing strains with reduced invasive potential escape nonopsonic neutrophil-dependent phagocytosis but, when they do translocate into the distal airway, are more easily cleared by AMs and more readily cleared in the lung or bloodstream by complement-dependent mechanisms. This may explain why they less commonly cause invasive disease. In contrast, the strains of greater invasive potential are readily cleared from the upper airway by neutrophils but if they escape neutrophil-mediated clearance they can more easily bind to epithelial cells via adhesins and translocate across the epithelium, as suggested by Weinberger and colleagues. They are also more resistant to AM- and complement-mediated clearance, explaining their relative success as invasive strains.

Strains associated with colonization have thicker capsules produced at lower metabolic cost. Serotype replacement is a well-recognized phenomenon after protein conjugate vaccination, and colonizing capsular serotypes that evolve with the selective pressure of vaccination are predicted to be those with the larger and less metabolically costly capsules.

Recognizing the Toxin Pneumolysin

A cholesterol-dependent cytolysin named pneumolysin is a critical virulence factor for pneumococci, but recent work has highlighted that both the direct effects of the toxin on cells and the immune response to the toxin may influence pathogenicity. Moreover, an increasing number of pattern-recognition receptors are shown to be involved in the innate immune response to pneumolysin. The capacity of pneumolysin to form membrane pores that lyse cells and to activate complement T cells, neutrophils, or macrophages at sites of infection is well established. Recent attention has focused on the pattern-recognition receptors involved in sensing pneumolysin.

Toll-like receptor (TLR) 4 was the first pattern recognition identified to interact with pneumolysin. However, not all innate responses could be linked to TLR4 engagement; a recent study identified TLR4-independent pneumolysin responses, including dendritic cell activation and production of interferon (IFN)-γ and IL-17A by T cells. This study noted the ability of pneumolysin to synergize with TLR engagement to stimulate cytokine production by macrophages and dendritic cells. Pneumolysin stimulated caspase-1-dependent processing of IL-1β, which is required to secrete the leaderless protein IL-1β. The authors observed PLY-dependent caspase-1 activation is dependent on the nucleotide-binding oligomerization domain (Nod)-like receptor (NLR) P3 inflammasome and the apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) adaptor protein (Fig 2), thus implicating a pattern-recognition receptor other than TLR4 in pneumolysin recognition. The authors also established a requirement for pore formation by pneumolysin and activation of the lysosomal protease cathepsin B for IL-1β release.

These results have been extended by another group, who showed that a number of strains of pneumococci that express a pneumolysin, which lacks pore-forming activity, are impaired in their capacity to induce NLRP3-dependent IL-1β secretion. These strains include the highly prevalent serotype 1 strain ST306, which causes empyema and disease outbreaks. These findings suggest that some strains’ success at causing invasive disease is related to their capacity to evade pattern-recognition receptors such as NLRP3. They also suggest, however, that the lower mortality associated with these strains might be linked to their lower potential to induce proinflammatory responses. The highly virulent serotype 1 strain 4496 (ST3018, lineage C) expresses pneumolysin of reduced hemolytic potential, which shares some of the mutations associated with other strains of reduced pore-forming activity but also has some unique mutations. In a murine model of invasive disease, a mutant-expressing pneumolysin from strain 4496 resulted in increased levels of bacteremia in the first 15 h of infection but also increased survival as compared with an isogenic mutant-expressing toxin of full hemolytic potential.
Figure 1. Pneumococcal virulence factors influencing colonization and invasion. CSTs and ISTs of Spn show distinct characteristics, which are in part defined by polysaccharide capsule. CSTs are more readily coated with COMP, but ADHs, including pneumococcal surface protein A, choline-binding protein A, and PsrP, are more accessible in ISTs. In the URT, the CSTs are likely to colonize in part because when nonopsonized they are less susceptible to PMN. In contrast, CSTs are readily phagocytosed by AMs in the LRT and are less likely to cause invasive disease. ISTs less readily colonize the URT and are more likely to adhere to ECs and penetrate tissue, ultimately reaching BVs and establishing bacteremia. ISTs are also more resistant to phagocytosis by AMs. Independent of the capsule, PsrP will favor biofilm formation and resistance to AM phagocytosis, whereas production of pili and PsrP will enable attachment to epithelia. Pili and PLY will also influence invasion and amplification of the inflammatory response. ADH = adhesin; AM = alveolar macrophage; BV = blood vessel; COMP = complement; CST = colonizing serotype; EC = epithelial cell; IST = invasive serotype; LRT = lower respiratory tract; PLY = pneumolysin; PMN = phagocytosis by neutrophils; PsrP = pneumococcal serine-rich repeat protein; Spn = Streptococcus pneumoniae; URT = upper respiratory tract.
In murine models, NLRP3-associated responses enhanced pulmonary clearance of pneumococci from the lung and moderately reduced lung injury and mortality.\textsuperscript{21,25} Another group have suggested that the impairment in IL-1β and IL-18 secretion and the host response to pneumolysin is more markedly perturbed in the absence of ASC, as opposed to NALP3, and have implicated a further inflammasome receptor that also uses ASC, absent in melanoma.\textsuperscript{2} In conclusion, NALP3 and potentially other inflammasomes have emerged as important mediators of host immunity to pneumococci. Strains that do not engage this recognition system may have a survival advantage that facilitates their spread into the bloodstream but, once in this location, their reduced capacity to generate inflammatory responses may result in improved survival.

**Additional Virulence Determinants**

Multiple other virulence factors contribute to pneumococcal pathogenesis.\textsuperscript{26,29} Two recent findings merit emphasis. PsrP is a pathogenicity island-encoded adhesin, which has emerged as contributing to lower respiratory tract virulence and in binding to the lung.

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**Figure 2.** Pattern-recognition receptors involved in the recognition of Spn. A variety of Spn-derived factors can stimulate TLRs expressed both on the cell surface and on phagolysosome membranes. TLR2 recognizes LPs, TLR4 PLY and TLR9 unmethylated CpG dinucleotides in bacterial DNA.\textsuperscript{22} The release of factors can be aided by digestion of bacterial components by lysosomal enzymes. In addition, some digested fragments may be released from a pPL and engage cytosolic pattern-recognition receptors such as MDP, which engages Nod2.\textsuperscript{23} PLY also activates the NALP3 INF containing the ASC adaptor protein,\textsuperscript{21} and potentially other inflammasomes, although the mechanism of release of these factors from the phagolysosome awaits clarification and it is not clear if the release is selective or part of a more generalized permeabilization event. Bacterial DNA (Spn DNA) released from the phagolysosome is recognized by DAI in the cytoplasm.\textsuperscript{24} ASC = apoptosis-associated speck-like protein containing a caspase recruitment domain; DAI = DNA-dependent activator of interferon regulatory factors; INF = inflammasome; LP = lipopeptide; MDP = muramyl dipeptide; NALP3 = nucleotide-binding oligomerization domain-like receptor; Nod = nucleotide-binding oligomerization domain; Nod2 = nucleotide-binding oligomerization domain-containing protein 2; pPL = permeabilized phagolysosome; TLR = toll-like receptor. See Figure 1 legend for expansion of other abbreviations.
CONTROL OF COLONIZATION

Upper airway colonization is thought to precede the development of pneumonia. Colonization is, however, a frequent event in comparison with pneumonia and in most cases does not lead to the development of clinical disease. T helper cell (Th)17 T-cell responses are key in clearing colonizing bacteria. The phagocytic effectors of Th17 T-cell-dependent bacterial clearance will, however, vary, depending on whether the host has been previously exposed to pneumococci. In naive mice, recruitment of macrophages aids clearance of colonizing bacteria, whereas in mice with prior colonization, neutrophils mediate more rapid bacterial clearance. Key events in the innate recognition of colonizing bacteria have also been defined. Nod2-mediated recognition of peptidoglycan fragments degraded by lysozyme M is critical for macrophage-mediated clearance of colonizing pneumococci and enables CCL2 chemokine production and recruitment of further macrophages. The macrophage response also requires the presence of pore-forming pneumolysin, and Davis and colleagues suggested that this could aid the cytosolic translocation of peptidoglycan fragments into the cytosol. TLR2, which recognizes lipopeptides expressed by pneumococci, made a smaller but additional contribution to CCL2 production. Nod2-dependent responses were also required for the development of Th17 responses and for adaptive immune responses to colonizing bacteria.

Type 1 IFN signaling reduces pneumococcal carriage because IFN-α/β receptor knockout mice are more heavily colonized with pneumococci compared with wild-type control mice. Type 1 IFN signaling is initiated by pneumococcal DNA that gains access to the cytosol via pore formation by pneumolysin and activates IFN-β production via the cytosolic sensor DNA-dependent activator of IFN regulatory factors. Influenza A virus triggers a synergistic enhancement of type 1 IFN to that produced during pneumococcal colonization, and this can inhibit the macrophage-driven CCL2 response required to clear colonizing bacteria. These studies illustrate how sensing of bacteria by a range of pattern-recognition receptors (eg, TLR2, Nod2, DNA-dependent activator of IFN regulatory factors) in the upper airway leads to Th17 and type 1 IFN responses and clearance of bacteria by macrophages and neutrophils. They also provide clues as to how viral infections can subvert innate responses to colonizing bacteria (eg, by inhibiting the early CCL2-dependent macrophage responses). TLR responses to colonization can, however, modify epithelial integrity, favoring translocation of bacteria. Recent work has shown that p38 mitogen-activated protein kinase–transforming growth factor-β-mediated responses lead to upregulation of SNAIL1, a transcriptional repressor of claudin-7 and claudin-10, which are required to maintain epithelial cell tight junctions. This illustrates the key concept that the inflammatory response must be carefully matched to the host’s requirements and that, although underproduction of key cytokines can hinder bacterial clearance, excessive production can favor tissue invasion and paresis of key effector functions, including phagocytosis.

OPTIMIZING MACROPHAGE EFFECTOR FUNCTIONS

Colonization and microaspiration of upper airway contents are frequent events, and clearance of colonizing bacteria can take a few weeks. The comparative infrequency of pneumonia suggests that efficient host responses must operate in the lower airway to protect against pneumonia. A number of components, including the cough reflex, the mucociliary escalator, and mucous and humoral factors in the lower airway, protect the airway from bacteria; however, cell-mediated host defense is critical. AMs are the resident phagocytes that clear bacteria from the lower airway and clear finite numbers of bacteria when these reach the lower airway. The capacity of the AMs to clear bacteria can be overwhelmed in the face of medical comorbidities that alter their clearance capacity for bacteria, such as COPD. They are also compromised by coexistent viral infections, in particular HIV. Factors such as cigarette smoking that result in alternative as opposed to classic activation of AMs also compromise the macrophages’ capacity to clear bacteria.

Differentiated tissue macrophages have a large lysosomal compartment and, when the killing capacity of AMs is exhausted, lysosomal permeabilization and activation of cathepsin D leads to apoptosis-associated killing of bacteria. Cathepsin D activation leads to a reduction in protein translation and enhanced
proteasomal degradation, with the result that levels of proteins with short half-lives, such as a key regulator of macrophage survival, the antiapoptotic protein Mcl-1, are reduced, allowing induction of AM apoptosis.\textsuperscript{37,38} Bacterial killing in AMs undergoing apoptosis involves reactive oxygen species (ROS) and nitric oxide (NO) and occurs at a stage when conventional microbicidal killing has become exhausted. Enhancement of the conventional mechanisms of intracellular killing, such as the generation of NO via inducible NO synthase, reduces the requirement for apoptosis-associated killing and can be achieved in animal models by administration of granulocyte-macrophage colony-stimulating factor.\textsuperscript{39} Thus, it seems that apoptosis-associated killing becomes essential in situations in which the macrophages’ capacity to control ingested bacteria is stressed and ensures a bacterial clearance process, minimizing the inflammatory response.\textsuperscript{37} Enhancement of the AM capacity to clear bacteria is, therefore, an attractive, but so far unexploited, aspect of the therapeutic approach to pneumonia.

**CONTROLLING NEUTROPHILIC INFLAMMATION**

If the initial inoculum of bacteria cannot be contained by AMs and other elements of the lung’s innate host defenses, then neutrophils are recruited to control bacterial replication. Macrophages and epithelial cells co-operate to generate CXC chemokines and other factors that activate a neutrophilic response (Fig 3).\textsuperscript{40,41} Although neutrophils are potent effectors of bacterial clearance, their recruitment results in the clinical features of pneumonia and runs the risk of causing lung injury. There is considerable scope, therefore, to manipulate neutrophilic inflammation during pneumococcal pneumonia. Neutrophil recruitment during pneumococcal infection requires the β2 integrin Mac-1, but not lymphocyte function-associated antigen, and also involves the α4β1 integrin (CD49d/CD29).\textsuperscript{43} Neutrophil killing of pneumococci does not require NADPH oxidase-dependent generation of ROS but is dependent on the generation of the neutrophil serine proteases cathepsin G and neutrophil elastase.\textsuperscript{44,45} In the absence of NADPH oxidase-generated ROS, mice tolerate greater numbers of neutrophils in the lung without a concomitant increase in lung injury.\textsuperscript{46}

In addition to modulation of neutrophil recruitment or generation of antimicrobial molecules, the neutrophilic inflammatory response might also be modulated by manipulation of the cytokine network. Deletion of myeloid-expressed phosphatase and tensin homolog deleted on chromosome 10 also reduces proinflammatory cytokine production and neutrophil recruitment, resulting in reduced lung injury and improved survival with pneumococcal pneumonia.\textsuperscript{47} Turning off proinflammatory cytokine responses at the appropriate time is also key to ensuring the appropriate resolution of neutrophilic inflammation. For example, an important role for triggering receptor expressed on myeloid cells-1 has emerged in both amplifying early inflammatory responses but also downregulating the later phase of the inflammatory response via upregulation of IL-1R-associated kinase M, a negative regulator of TLR signaling.\textsuperscript{48} Matrix metalloproteases 2 and 9 cleave IL-17A, also reducing neutrophil recruitment to the lung.\textsuperscript{49}

A further critical part of the resolution phase of pneumococcal pneumonia is the clearance of apoptotic neutrophils by macrophage efferocytosis. In addition to enhancing neutrophil function, galectin 3 enhances macrophage efferocytosis and ensures less lung damage arises during pneumococcal pneumonia.\textsuperscript{50}
The manipulation of neutrophilic inflammation is an important goal because a subset of individuals with pneumococcal pneumonia die with a dysregulated inflammatory response despite complete clearance of bacteria.

THE ROLE OF T CELLS IN PNEUMONIA
Pathogenesis

The adaptive arm of the immune response is also critical to the host response to pneumococci. B cells are critical for antibody responses to naturally occurring infection and immunization, but review of their role is beyond the scope of this article. T cells contribute to the pathogenesis of pneumococcal pneumonia. T-cell numbers increase in the peribronchiolar region early during the evolution of pneumonia. Nevertheless, the exact role of T cells in pneumococcal pneumonia has been debated. Recent experiments suggest that CD4+ T-cell numbers or activation may adversely impact the cytokine network and survival during pneumococcal pneumonia. Conversely, CD8+ T cells may play an important role, regulating Th17 T-cell numbers in the lung, production of CXC chemokines, and neutrophil recruitment, and in their absence, survival is impaired during pneumococcal pneumonia. Nevertheless, a certain level of CD4+ T-cell responsiveness is required for an optimal response to pulmonary pneumococci in many settings; Th17 responses improved bacterial clearance in immunized mice and monocytes primed Th1/Th17 responses to pneumococci via IL-12 production. These findings suggest that the composition and activation state of T-cell populations must be tightly regulated in the early stages of pneumonia to maximize bacterial clearance but to ensure that the inflammatory response is kept to a minimum. Invariant natural killer T cells are also emerging as contributing to the host response to pneumococci and, following IL-12 or glycolipid-driven activation, contribute to IFN-γ production and bacterial clearance, enhancing early microbial clearance and limiting inflammation.

PERSPECTIVE

The ongoing evolution of pneumococci will continue to challenge immunization protocols. An improved understanding of how early immune responses can be enhanced and how neutrophilic inflammation can be more tightly regulated will lead to the development of more rational therapeutic strategies. In order for this to occur, the wealth of information derived from basic science and animal studies needs to be translated to patient groups, and novel therapeutic approaches manipulating the host response must be developed, building on these findings. Areas of attention should include building a more accurate molecular understanding of the basis of susceptibility. This can be achieved through application of findings relating to the early steps in the immune response to subclinical infection and the early stages of pneumonia. Such knowledge could be used to better stratify patients who are at risk for enhanced immunization programs or other preventive strategies. In addition, a better understanding of the core components of a successful inflammatory response to pneumococcal pneumonia is needed. This can inform therapeutic approaches that maximize bacterial clearance but minimize the inflammatory consequences both to the lung and systemically. These aims may seem aspirational, but the constant evolution of the pneumococcus demands that we develop innovative approaches to combat a major global cause of medical illness.

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