The role of Adhesion Moleculesin T cell Activation, Immunotherapy, and Adhesin-Based Vaccines to Block attachment and colonization of Antigens

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Abstract: Mucosal immune dysfunction include infections, dietary indiscretions such as excess refined sugars and lack of fiber, allergies and food intolerances, indiscriminant and overuse of oral antibiotics, disruption of lipid and fatty acid metabolism, and aging. All of these etiologies, most a product of modern lifestyle, can result in dysfunctional mucosal immunity.

Damage to the mucosal barrier is significantly more sophisticated than merely the loosening of tight junctions, referred to as "leaky gut syndrome. "In fact, viruses utilize sophisticated chemical transport systems to gain entry into the interior of the body.

Several studies suggest that, Adhesions are antigen cell surface molecules or structures that mediate the attachment of the antigen to host cells and thus the host–pathogen interaction. adhesions and their role in attachment to host cells leads to the triggering of immune responses, which are crucial to the host's defence against infection

On the contrary, studies suggest that, large number of different adhesin genes in an organism's genome has multiple pathways for antigen adherence. This redundancy limits the use of adhesins as vaccine candidates, but normal mucosal immunity function and APCs a breakthrough in the use of adhesins as potential vaccine candidates.

In this article, I will describe, Adhension Signaling in T cell Activation, Mechanisms of Colonization and Invasion, Cell-Based Immunotherapy and Tumor Vaccines, NK Cell-Based Immunotherapy, and Adhesin-Based Vaccines to Block attachment and colonization of Antigens

Key Words: Antigens,Adhesions,NK Cell,T cell, Immunotherapy, and Vaccine,

The Content of Table

1. Introduction

Adhesion molecules (AM) are involved in many fundamental processes of the cell. They are closely associated with cytoskeletal filaments and signal molecules in the cell and with AM of the basement membrane, extracellular matrix (ECM) or adjacent cells. AM are transmembrane glycoproteins acting as a molecular link between the outside and inside of the cell. They have three domains: the extra-cellular domain bound to the ECM or adjacent cell membrane, acts as a signal receptor from outside. The central domain traverses the cell membrane and the intracellular part transports signals being attached to various ligands - cytoskeletal proteins, enzymes or similar molecules that can link to metabolic pathways within the cell, or to transcription factors. The complex ligand - transcription factor may enter into the nucleus and bind to DNA(1).There are several families of AM: (a) Immunoglobulin-like superfamily, (b) Cadherins, (c) Integrins, (d) Receptor protein tyrosine phosphatases (RPTP), (e) Selectins, (f) Hyaluronate receptors and (g) Dipeptidyl peptidase IV (CD26).

2. Adhension Signaling in T cell Activation

To be fully activated the T cell must sustain signaling for many hours. The reasons for this prolonged requirement for TCR-mediated signals may relate to passing certain cell-cycle checkpoints and possibly also to the time required for chromatin remodeling to activate cytokine gene expression(2).There are two alternative strategies to achieve sustained signaling that both lead to T-cell proliferation. One is the formation of a stable immunological synapse in which the T cell remains with one APC for the duration of primary stimulation. The other is the formation of a series of transient synapses with multiple APC over the same time period. We consider the molecular mechanisms involved in each type of interaction and how these different modes ofstimulation can each result in T-cell proliferation.
The immunological synapse is a highly polarized structure that allows coordination of T-cell migration with the antigen recognition process, sustained signaling, and bidirectional

communication with the APC. The immunological synapse has a bull's-eye pattern with a central cluster of TCR surrounded by a ring of LFA-1, a major integrin adhesion molecule involved in T cell– APC interactions. Secretory granules and the Golgi apparatus are focused on the central cluster to maintain a focused cytokine-mediated communication with the APC(3).The organization of the immunological synapse may even be mapped onto the classical polarized systems–the epithelial cell. In this analogy, the adhesion ring would correspond to the basolateral surface and the central cluster would correspond to the apical surface.
There is no direct correlate of the tight junction complex, but the demarcation between the central cluster and the ring is very sharp and probably involves molecular specialization to prevent mixing of these compartments. The adhesion ring is itself surrounded by a very active ruffling membrane. The ruffling membrane suggests ongoing activation of rac family G-proteins that interact with downstream molecules to stimulate membrane ruffling(4).The generation of this pattern involves the initial formation of TCR/MHC–peptide interactions in the periphery of the contact, which are then transported to the central region. This radial inward movement also reflects the polarization of the T cell toward the APC.
Immersing the T cell–APC interaction in a collagen gel breaks the stable polarization between the T cell and the APC, in which case the T cell forms more transient interactions with a series of APC over time(5).These sequential interactions are likely to result in similar compartmentalization of signaling as the stable immunological synapse and can also lead to proliferation, but the coordination of migration and antigen recognition is lost. Interestingly, collagen is sequestered from T cells in secondary lymphoid tissues(6).Current evidence suggests that the T-cell area of the lymph node is a high-density cell suspension in interstitial fluid with a specific structure supplied by the reticular fiber network: the three-dimensional environment of the T cell. For this reason we favor the idea that primary T-cell activation in lymph nodes will operate through the formation of a stable immunological synapse(7).In contrast, effector interactions in collagen-rich tissues may operate in a serial mode much like the classical model for serial killing of targets by CTL(8).
The immunological synapse coordinates adhesion, signaling, and cytoskeleton. Immunological synapse formation requires an intact actin cytoskeleton and it has been suggested that it is a classical case of tension generation by myosin II contraction of an actin filament network(9),(10).The trigger for this process is signaling through the TCR and LFA-1, with cross-talk between these spatially distinct signaling regions in the synapse(11).It is well known that TCR signaling rapidly activates adhesion through changes in LFA-1 activity(12),(13).The mechanism of this process is not known with certainty, but candidates include the branches of the mitogen-activated protein kinase pathway and the regulation of actin cytoskeleton through Syk-family tyrosine kinases and the Vav exchange factor(14).While the details of this signaling pathway are not known, it may also involve activation of the small G-protein Rap1(15),(16),(17).Physiological TCR engagement and activation of adhesion pathways are likely to be totally interdependent processes that cannot be separated. Nonetheless, signaling processes appear to be compartmentalized (Fig. 2). The TCR signaling pathway controls the activation of three tyrosine kinase families, the src, syk, and itk families, and a lipid kinase, phosphatidylinositol-39- kinase (PI-3-K). These kinases are assembled together around a tyrosine phosphorylated scaffold formed by the TCR-associated CD3 g, d, «, and z subunits and the linker of activated T cells (LAT). Critical downstream pathways include activation of the mitogen-activated protein kinase and Jun kinase pathways and NFkB and NFAT transcription factors that are required for full T-cell activation. The ability of the T cell to suppressTCR-mediated death signaling is carried out by costimulatory receptors like CD28 and

ICOS(18),(19),(20).These survival receptors augment activation of PI-3-K, and the generation of phosphatidylinositol-3,4,5-trisphosphate augments recruitment and activation of the pleckstrinhomology (PH) domain containing kinases like Itk and Akt (also known as protein kinase B)(21).Akt is a serine/throenine kinase that activates or inhibits downstream molecules to promote T-cell survival(22).CD28 looks like an adhesion molecule that might be expected to activate PI-3-K in response to T-cell contact with any ligand-expressing cell, but this may not be the case *invivo*. CD28 engagement is tightly controlled by TCR engagement to a greater extent than adhesion molecules LFA-1 and CD2. CD28 does not appear to augment TCR engagement(23),but rather it appears to be the other way around. Thus, it is appropriate to classify CD28 in aseparate category from LFA-1 and CD2. It remains to be seen whether ICOS is similarly regulated. Signaling from the adhesion ring is also likely to play a key role in cell-cycle progression. The bestcharacterized biochemical pathway is based on activation of the Jun/Fos transcriptional coactivator JABI by LFA-1(24).Sustained LFA-1 engagement in conjunc- tion with IL-2 receptor engagement also down-regulates the cell-cycle inhibitor p27kip, thus enabling movement from the G1 to the S phase of the cell cycle(25).Another interesting property of the LFA-1 ring is that it is relatively depleted of the transmembrane tyrosine phosphatase CD45. CD45 has recently been shown to act as a Janus kinase phosphatase and thus a negativeregulator of cytokine signaling pathways (25).To enter the cell cycle the T cell must integrate TCR, survival, and cytokine signals. It is attractive to speculate that the LFA-1 ring may be a site of active cytokine signaling both due to the depletion of CD45 and by analogy with focal adhesion complexes that also have a well-known role in sustaining growth factor signals(26).

3. The structure and functions of Ig-like cell-adhesion molecules

An intriguing aspect of viral receptor usage is the widespread exploitation of cell-surface glycoproteins that are found predominantly in intercellular junctions of polarized cells. Perhaps the most widely used class of adhesion molecule is the IgSFCAMs . The immunoglobulin-like superfamily of proteins is characterized as consisting of seven to nine anti-parallel beta-strands that form two beta-sheets in a Greek-key motif, having a barrel shape. The superfamily is subdivided according to the number of beta-strands andtopological similarities to the constant (c) or variable (v) chains of antibodies (V, C1, C2, I). Figure 1a showsthe topology of the V-set Ig-like fold. JAM-A is a prototypic tight junction associated IgSF CAM expressed on epithelial and endothelial cells as well as on leucocytes and platelets. It comprises two extracellular Ig-like domains, a single transmembrane region and a short cytoplasmic tail. X-ray crystallography of an ectodomain soluble fragment of human JAM-A reveals that the N-terminal, membrane- distal D1 domain has nine beta-strands and is therefore classified as similar to the antibody variable domain (V-set).
The membrane proximal D2 domain, on the other hand, is classed as I-set, having only eight strands (figure 1b). Analytical ultracentrifugation analysis of recombinant soluble murine JAM-A revealed that it forms homodimers. Both murine and human JAM-A crystal structures show a similar non-covalent interaction between the membrane-distal D1domains at the GFCC' face, and this is thought to represent the dimeric state of JAM-A at the cell surface.Homotypic and heterotypic interactions with JAM-A and other adhesion molecules, respectively, on adjacent cells, are then thought to regulate tight-junction formation and facilitate leucocyte transmigration.

4. Function of cell adhesion molecules in development, motility and migration

The adhesion molecules are involved in the cell differentiation, migration and sorting. The most direct effect of cell-cell adhesion is on morphogenesis, that is, on the assembly of individual cells into highly ordered tissues and organs. Sequential expression of cell adhesion molecules on the surface of embryonic cells is essential for early organisation of the developing embryo. E-cadherin is one of the first adhesion molecules that human express. Cadherins regulate a variety of early events, such as the interactions between ectoderm and mesoderm that lead to formation of the neural plate. T cadherin is absent from muscle cells during early development and is upregulated once innervation has occurred and synapses formed. N cadherin anchors the nerve to the synaptic site. CD44 takes place in the developement of the limb: antibodies to CD44 block cartilage formation.
Cell adhesion molecules play important role in controlling cell migration through their connections with cytoskeleton. It was mentioned that cadherins associate with catenins which are attached to contractile molecules such as actin. Cell motility across a matrix is regulated through cell attachment and detachment mediated through a cell adhesion molecules. Cells extend cytoplasmic protrusions called invadopodia, that become tightly adherent at its tip. For this to occur, cells need to cause migration and aggregation of cell adhesion molecules within their cell membranes. The α3 β1 and α5 β1 integrins play major organisation roles in the adhesion and formation of invadopodiapromoting invasive cell behavior (27). Cell surface proteases (e.g. CD26)also play an important role in facilitating cell invasion into the ECM(28).αVβ3integrins has been shown to modulate ECM proteolytic activity by recruiting a majorsoluble proteinase, matrix metallo-proteinase–2 (MMP-2), to the cell surface.

5. The Role of Adhesins in Microbial Pathogenesis

To initiate infection, bacterial pathogens must first be able to colonize an appropriate target tissue of the host(28),(29).This tropism (ability to gain access to a niche within the body),in association with the ability of the bacterium to breach mucosal barriers and invade the host,distinguishes pathogenic from commensal organisms. Colonization begins with the attachmentof the bacterium to receptors expressed by cells forming the lining of the mucosa Certain species of bacteria are restricted interms of the hosts and tissues they infect and the diseases they cause. In many cases, tropism for specific tissues has been corroborated in the laboratory by in vitro binding assays with isolated epithelial cells collected from sites ofinfection or from infection-prone hosts. Attachment is mediated by adhesin proteins;bacterial lectins are the most common type of adhesin among both gram-negative and grampositive bacteria(30),(31).Adhesins, such as theFimHadhesin produced by most Enterobacteriaceae (including uropathogenic E. coli), are highly conserved proteins(32).This lack of major variation is most likely due to the requirement that all pathogenic strains recognize invariant host receptors. Although minor changes in the adhesin protein have been observed (2% divergence) and correlate with decreased or increased affinity for binding to sugars(33),antibodies against a single FimH protein crossreact with >90% of E. coli strains expressing the FimHadhesin and block binding to bladder cells in vitro(34),(35).Furthermore, antibodies against FimH from a single isolate protect against in vivo colonization by >90% of uropathogenic strains in a murine

model for cystitis (unpub. obs. This high degree of antigenic conservation is another reason why adhesins may serve as ideal vaccines. Aside from mediating colonization of the host, bacterial attachment often results in the up-regulation or expression of many other virulence genes encoding various proteins that allow for invasion of the host(36),(37),(38),(39),(40),(41),(42). The proteins can mediate tighter associations with epithelial cells, trigger epithelial cell.actin filament rearrangements, and induce changes in host-cell signaling and function. In some cases, the bacteria may also secrete a protein that inserts into mammalian cells and serves as a receptor for its own intimate adherence with the host (43).Given that cross-talk between pathogenic bacteria and host cells after microbial attachment may trigger expression of virulence factors leading to local inflammation or invasive disease, vaccines that block bacterial attachment may have multiple advantages. Many studies have demonstrated the utility of vaccines against bacterial surface components in blocking attachment in vitro as well as in vivo(44),(45),(46),(47). However, as understanding of the mechanisms of attachment has evolved and characterization of specific adhesin molecules has been refined, new opportunities have emerged for the development of adhesin-based vaccines.

6. Mechanisms of Colonization and Invasion

6.1 Adhesins and Receptors

Adherence to skin or mucosal surfaces is a fundamental characteristic of the normal human microflora and also an essential first step in the pathogenesis of many important infectious diseases. Most microorganisms express more than one type of adherence factor or “adhesin.” a large fraction of microbial adhesins are lectins that bind directly to cell surface glycoproteins, glycosphingolipids, or glycosaminoglycans; adhesion may be mediated through terminal sugars or internal carbohydrate motifs. In other cases, the bacteria express adhesins that bind matrix glycoproteins (e.g., fibronectin, collagen, or laminin) or mucin, providing a form of attachment to the mucosal surface. The specific carbohydrate ligands for bacterial attachment on the animal cell are often referred to as adhesin receptors and they are quite diverse in nature. The tropism of individual bacteria for particular host tissues (e.g., skin vs. respiratory tract vs. gastrointestinal tract) is effectively determined by the array of available adhesin-receptor pairs.

In a number of cases, the key adhesive factor is an assembly of protein subunits that project from the bacterial surface in hair-like threads known as pili or fimbriae. Such pili are usually composed of a repeating structural subunit providing extension and a different “tip adhesin” that actually mediates the host-cell interaction. Often the structural genes and enzymes for pilus assembly are encoded in a bacterial operon. Lateral mobility of pili structures in the bacterial membrane provides a Velcro-like binding effect to epithelial surfaces. Certain strains of E. coli express pili that bind avidly to P-blood group-related glycosphingolipids in the bladder epithelium, leading to urinary tract infection. Pathogenic strains of Salmonella produce pili that facilitate adherence to human intestinal cell mucosa, thereby causing food poisoning and infectious diarrhea. In other cases, a surface-anchored protein (afimbrialadhesin) expressed by the bacteria represents a critical colonization factor. For example, the filamentous

hemagglutinin (FHA) of Bordetella pertussis promotes strong attachment of the bacteria to the ciliated epithelial cells of the bronchi and trachea, triggering local inflammation and tissue injury that results in the syndrome of "whooping cough." FHA is a component of modern pertussis vaccines given in infancy and early childhood to block infection. For certain pathogens, epithelial attachment is a two-step process, in which a microbial glycosidase acts upon a target cell polysaccharide to modify its structure into a novel glycan, which then serves as the adhesin receptor. For example, a secreted P. aeruginosa neuraminidase produces increased numbers of asialoglycolipid receptors, which may promote colonization of the cystic fibrosis airway. Likewise, the neuraminidase of S. pneumoniae removes sialic acid from respiratory epithelial cells to expose underlying N-acetylglu-cosamine and galactose residues to which the bacterium binds with higher affinity.

6.2 Invasion Factors

Glycan–lectin interactions play pivotal roles in enabling certain pathogens to penetrate or invade through epithelial barriers, whereupon they may disseminate through the bloodstream to produce deep-seated infections. S. entericaserovarTyphi causes typhoid fever in humans, a process that begins with intracellular invasion of intestinal epithelial cells. The outer core oligosaccharide structure of the serovarTyphi LPS is required for internalization in epithelial cells. Removal of a key terminal sugar residue on the outer core markedly reduces the efficiency of bacterial uptake. Streptococcus pyogenes, the common cause of strep throat but also an agent of serious invasive infections, attaches to human pharyngeal and skin epithelial cells through specific recognition of its hyaluronan capsular polysaccharide by the hyaluronan-binding protein CD44. This binding process induces marked cytoskeletal rearrangements manifested by membrane ruffling and opening of intercellular junctions that allow tissue penetration by GAS through a paracellular route. The human malaria parasite Plasmodium vivax is completely dependent on interaction with the Duffy blood group antigen for invasion of human erythrocytes. The Duffy blood group antigen is a 38-kD glycoprotein with seven putative transmembrane segments and 66 extracellular amino acids at the amino terminus. The binding site for P. vivax has been mapped to a 35-amino-acid segment of the extracellular region at the amino terminus of the Duffy antigen. Unlike P. vivax, P. falciparum does not use the Duffy antigen as a receptor for invasion. A 175-kD P. falciparum sialicacid-binding protein (also known as EBA-175 [erythrocyte-binding antigen-175]) binds sialic acid residues on glycophorin A during invasion of the erythrocyte. Some P. falciparum laboratory strains use sialic acid residues on alternative sialoglycoproteins such as glycophorin B as invasion receptors, with binding being mediated by other EBA family members. The use of multiple invasion pathways may provide P. falciparum with a survival advantage when faced with host immune responses or receptor heterogeneity in host populations.

Biofilm formation is another mechanism that promotes bacterial attachment to host surfaces, often in the form of a polymicrobial community. For example, oral biofilms comprise, in total, about 1000 species, only half of which are culturable and the remaining species can only be

identified by nucleic acid detection methods. Streptococcus species predominate (60–90%), but Eikenella, Haemophilus, Prevotella, and Priopionibacterium species can also be found. Dental plaque represents an oral biofilm in which dense, mushroom-like clumps of bacteria pop up from the surface of the tooth enamel, interspersed with bacteria-free channels filled with extracellular polysaccharide (EPS) produced by the bacteria that can serve as diffusion channels. Bacteria within biofilms communicate with one another through soluble signaling molecules in a process known as "quorum sensing" to optimize gene expression for survival. In biofilms, bacteria live under nutrient limitation and in a dormant state in which defense molecules (e.g., antimicrobial peptides) produced by the immune system and pharmacologic antibiotics are less effective. Moreover, the EPS matrix can bind and inactivate these same agents, contributing to the persistence of the biofilm and difficulty in medical treatment of biofilm infections, such as those that arise on catheters and other medical devices. The EPS synthesized by biofilm bacteria vary greatly in their composition and in their chemical and physical properties. The majority of EPS types are polyanionic because of the presence of either uronic acids (D-glucuronic, D-galacturonic, or D-mannuronic acids) or ketal-linked pyruvate. Inorganic residues, such as phosphate or sulfate, also contribute to the negative charge. In rare cases, EPS is polycationic, as exemplified by the adhesive polymer obtained from Staphylococcus epidermidis strains that produce biofilms on catheters. Ordered secondary configuration frequently takes the form of aggregated helices. In some of these polymers, the backbone composition of sequences of β1-4 or β1-3 linkages confers rigidity (as is seen in the cellulosic backbone of xanthan from Xanthomonas), whereas the β1-2 or β1- 6 linkages found in many dextrans provide more flexible structures. It is thought that the EPS itself can serve as the primary carbon reserve for biofilm microorganisms during substrate deprivation.

7. Adhesins as Vaccines: FimH as a Paradigm for Adhesin-Based Vaccines to Block Colonization

One of the key aspects of proving the potential efficacy of an adhesin-based vaccine in vivo is the development of an animal model of disease that relies on bacterial colonization of the mucosal epithelium mediated by the specific adhesin of interest. Although seemingly straightforward, testing for protection in small animal models of disease is difficult for various reasons: large doses of in vitro grown bacteria are required to establish mucosal colonization in animals, which does not necessarily mimic the course of infection in humans; specific glycoprotein receptors for some adhesins are lacking in animal mucosal tissues that correspond to the site of colonization in humans; and some bacterial adhesins that are usually expressed as part of a larger structure on the bacterial cell surface (e.g., tip adhesins associated with whole pili) are difficult to purify. Despite these difficulties, adhesin-based vaccines have demonstrated efficacy in protecting against infection, thus proving the usefulness of such molecules as subunit vaccines. Research using the FimHadhesin from E. coli provides one such example. Type 1 pili have long been implicated in bacterial urinary tract infections in humans(48),(49).In a murine cystitis model, colonization of the bladder by E. coli was shown to depend ongrowth conditions that favored expression of type 1 pili and in particular required FimH(34),(50).Thus, the murine model was a valid small-animal model to prove whether adhesinbased vaccines might block colonization. Although purifying large amounts of pilusassociatedadhesin is

difficult (because most adhesins are proteolytically degraded when expressed as independent moieties), Hultgren et al. demonstrated that the FimHadhesin could be stabilized in an active conformation by the periplasmic chaperone FimC, making it possible to purify full-length FimH protein. Vaccination with the FimCH complex elicited long-lasting immune responses to FimH. Sera from mice vaccinated with the FimH vaccine inhibited uropathogenic strains of E. coli from binding to human bladder cells in vitro. Vaccination with the FimHadhesin-vaccine reduced in vivo colonization of the bladder mucosa by >99% in the murine cystitis model(34).Furthermore, the FimH vaccine protected against colonization and disease by uropathogenic strains of E. coli capable of expressing multiple adhesins. IgG specific for FimH was detected in the urine of protected mice, consistent with our originalhypothesis that antibodies directed against an adhesin protein might protect along the mucosalsurface. Subsequent studies in a primate model of cystitis have corroborated these findings(Langermann et al., unpub. data). Furthermore, in primate studies we demonstrated a directcorrelation between the presence of inhibitory antibodies in secretions and protection againstcolonization and infection. While IgG antibodies elicited against adhesins are protective, induction of immune responses along the mucosa can be augmentedby a variety of antigen delivery systems that specifically target mucosa-associated lymphoidtissue and activate the mucosal immune system(51).These delivery systems include wholeinactivated or live-attenuated bacterial and viral vectors, biodegradable microspheres, liposomes, transgenic plants, and antigens conjugated to or coadministered with the cholera toxin B subunit or attenuated forms of heat labile toxin from E.coli. Many of these systems hold promise for future vaccine strategies, but only a few have been tested in humans for safety and adjuvanticity. As these mucosal adjuvants progress further toward approval for use in humans, testing should be done with adhesion antigens to determine if induction of local immune responses enhances the protective efficacy of adhesin-based vaccines as compared with conventional parenteral vaccination. Such studies are under way with the FimH vaccine. Given the preclinical data with the FimH vaccine, similar efforts should be directed at developing adhesin-based vaccines for a wide range of pathogens. In this regard, additional efforts should also be focused on developing mucosal models of infection. The availability of such models should allow for appropriate screening of adhesin-based vaccines to prevent infections by streptococci, staphylococci, and other pathogens for which vaccine coverage is absent or inadequate.

8. Cell-Based Immunotherapy and Tumor Vaccines

Several T-cell-based immunotherapy strategies are under investigation, such as autologous/allogeneic transplantation of tumor specific CTLs, oncolyticvirotherapy, allogeneic NK cell infusions, and tumor vaccines(52),(53).Adoptive immunotherapy with autologous and/or allogeneic cancer antigen specific CTL has been investigated in both solid and hematologic cancers(54).Oncolyticvirotherapy uses attenuated viruses targeted to specifically infect host cancer cells leading to direct antitumor effects and immunologic cell death from tumor antigen presentation(55).Killer immunoglobulin- (KIR-) mismatched NK cell infusions are currently under investigation for pediatric leukemias, neuroblastoma, and

sarcomas(56),(57).Several tumor vaccine approaches have also been studied in pediatric cancer, using peptide alone or DC pulsed with tumor peptides or lysates. EBV-associated posttransplantlymphoproliferative disease (PTLPD)/lymphoma was treated by infusing donorderived EBV-specific CTL generated using EBV-transformed lymphoblastoid B-cell lines. Infusion of EBV-specific T cells after SCT was found to be highly effective to prevent the development of PTLD and treat preexisting disease(58),(59),(60). EBV-associated tumors express viral antigens and can be targeted using EBV-specific CTL. The association of pediatric nasopharyngeal carcinoma with EBV makes EBV antigens an immunotherapeutic target for cell-based therapy. Several ongoing and recently completed trials utilize either autologous or most closely HLA-matched EBV-specific (LMP-1 and LMP-2) CTL to treat nasopharyngeal carcinoma (NCT00953420, NCT01447056, and NCT00516087).

9. NK Cell-Based Immunotherapy

The antitumor effects of NK cells make them a potential immunotherapy option in the postallogeneic hematopoietic transplant setting and also in the nontransplant setting. Adoptive therapy with NK cells has been carried out to treat AML and several solid tumors, including ovarian cancer, melanoma, breast cancer, renal cell cancer, and advanced lung cancer(60),(61),(62),(63).Due to relatively low numbers in the peripheral blood, immunotherapy with NK cells requires ex vivo expansion to achieve clinically relevant numbers, and, during the expansion process, these cells display increased expression of activation markers, chemokine receptors, and adhesion molecules(64),(65),(66).Further, these studies using ex vivo activated and expanded NK cells have demonstrated extensive cytotoxicity against various tumor cells without affecting the healthy cells. Two pediatric trials (NCT01875601 and NCT01944982) employing ex vivo activated and expanded allogeneic NK cells are ongoing and one trial (NCT00640796) has been recently completed (in 2014). A pilot study on 10 children with AML employed the use of nonactivated, KIR/KIR ligand mismatched haploidentical donor NK cells along with exogenous IL-2 with all 10 subjects remaining in remission 2 years after infusion(67).A similar study using NK cells in adults reported complete remission in 75% of subjects with KIR/KIR ligand mismatches(62),(68).There are more than 20 open or recently completed clinical trials employing NK cell-based immunotherapy for pediatric cancers as reported recently in a comprehensive review by McDowell and coworkers(68).These studies either employ NK cells as monotherapy or in combination with chemotherapy and/or a mAb, such as anti-GD2 antibody. A recent study by Rubnitz and coworkers reported that 76% of children with relapsing or refractory leukemia treated with chemotherapy followed by the infusion of haploidentical NK cells proceeded to hematopoietic cell transplantation and 31% were alive when compared to a parallel study conducted by the same group with only 13% of patients alive with the same chemotherapy, but without NK cells(69),(70).

10. Conclusions

The key signaling features of the immunological synapse that distinguish it from earlier studied phenomena such as antibody-mediated capping is the requirement to coordinate cell– cell adhesion and the signaling process. The actin cytoskeleton plays a substantial role in bridging these singling systems at the membrane. The combination of ch14.18 antibody, aldesleukin, and GM-CSF is a prime example of how mAbs can have a significant impact on patient survival and the notion that immune strategies can safely be incorporated into our standard chemoradiation approach. Therefore, the combination of immunotheraphy with vaccine benefits normal mucosal immunity function and APCs activation that can be a breakthrough in the use of adhesins as potential vaccine candidates.

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