The Pathogenesis of Multiple Myeloma: Understanding the Mechanisms of the Bone Marrow Microenvironment

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Authors' disclosures of potential conflict of interest are found at the end of this article.

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Abstract

Multiple myeloma, a mature B-cell malignancy, is the second most common hematologic malignancy in the United States. Research has highlighted the importance of the bone marrow microenvironment in contributing to the pathogenesis of multiple myeloma via a network of complex interactions. As a result, novel agents have been developed that target the cellular and molecular mechanisms of the disease. In order to prescribe novel agents appropriately, monitor for response, and manage adverse effects, advanced practitioners will need to keep abreast of all the new molecules, signaling pathways, and relevant interactions that occur in the bone marrow microenvironment contributing to myeloma. This article will provide a review of the differentiation pathway of B lymphocytes into plasma cells, the cellular and molecular anatomy of the bone marrow microenvironment, as well as the most current findings of how the myeloma cell impacts the bone marrow microenvironment.

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Multiple myeloma (MM) is a mature B-cell malignancy that results in the clonal expansion of neoplastic plasma cells (Huff & Matsui, 2008). It is characterized by an overproduction of monoclonal proteins (M proteins), the formation of lytic lesions, and an accumulation of malignant plasma cells in the bone marrow (O'Connor, Gleeson, Noelle, & Erickson, 2003; Huff & Matsui, 2008). The transformation of a malignant plasma cell is considered to be a multistep process that is closely tied to the normal B-cell differentiation pathway (O'Connor et al., 2003). Additionally, numerous studies have highlighted the importance of the bone marrow microenvironment in contributing to the pathogenesis of MM (Hallek, Bergsagel, & Anderson, 1998; Podar, Chauhan, & Anderson, 2009). In the initial stages of the disease, myeloma cells migrate to the bone marrow and establish synergistic relationships with numerous cellular, noncellular, and liquid components. As a result, the natural homeostasis of

the microenvironment is disrupted via alterations in cytokine regulation, expression of cell surface receptors, and triggering of key signaling pathways, resulting in an environment that supports the growth and survival of myeloma cells (Billadeau, Ahmann, Griepp, & Van Ness, 1993; Shain et al., 2009; Pratt, 2002).

Novel agents have been developed targeting these cellular and molecular mechanisms of disease. Additionally, ongoing research continues to uncover new molecules, signaling pathways, and relevant interactions that occur in the bone marrow microenvironment (BMME), contributing to myeloma. An in-depth and *current* understanding of the processes of B-cell differentiation as well as the BMME will provide advanced practitioners (APs) with the knowledge base needed to prescribe novel agents appropriately, manage adverse effects, and monitor for disease response. Additionally, as new agents emerge from clinical trials, APs will be able to identify and comprehend the cellular and molecular targets being tested. Therefore the purpose of this article is to provide a review of the maturation process of a B cell to a plasma cell and the cellular and molecular anatomy of the BMME. This will be followed by a discussion of how myeloma cells alter the normal homeostasis of the BMME and acquire critical properties, such as immortality, that contribute to the growth and progression of the disease.

Immune System Review

The purpose of the immune system is to protect an individual from pathogens and infections. It has the capacity to distinguish self from nonself, and accomplishes this through a large repertoire of cells and molecules that collectively recognize foreign invaders and coordinate a response to eliminate them (Abbas, Lichtman, & Pober, 2000). The immune system can be classified into two separate systems: innate immunity and adaptive immunity. Innate immunity is considered to be nonspecific to antigens and is primarily designed to prevent infections via barriers (skin, mucosa, epithelium) or eliminate infections via phagocytosis (macrophages, neutrophils). In contrast, adaptive immunity is highly specific, capable of recognizing and eliminating specific antigens, and has the ability to remember and respond with repeated exposures (Kindt, Goldsby, & Osborne, 2007). The adaptive immune response is comprised of cell-mediated immunity, which is primarily performed by T lymphocytes, and the humoral response, which is mediated by B lymphocytes and antibodies (Abbas et al., 2000).

HUMORAL IMMUNE SYSTEM

Humoral immunity is provided via antibodies (immunoglobulins) that are produced by B lymphocytes. The function of antibodies is twofold: (1) to bind to the antigen or pathogen to "mark" it for elimination and (2) to orchestrate other cells and molecules to eliminate the marked antigen (Abbas et al., 2000). Antibodies are proteins that exist in two forms: membrane bound on B lymphocytes (antigen receptors) and as soluble molecules secreted by plasma cells (Kindt et al., 2007c). There are over 1 trillion B lymphocytes in the body and each one is specific to a single antigen (Alberts et al., 2002). It is this specificity that provides for the recognition portion of the immune response. Immunoglobulins will remain membrane bound until the B cell becomes activated and transforms into a plasma cell. The plasma cell in turn has little or no membrane-bound antibody but instead produces large amounts of the soluble form. Plasma cells are highly specialized and capable of secreting a few hundred to 1,000 molecules of antibodies per second. They represent the final stage of a B cell and are not capable of self-renewal, living only a short life span of days to weeks (Hallek et al., 1998; Kindt et al., 2007a). A very small percentage of plasma cells will become long-lived plasma cells and return back to the bone marrow. The process of how a B cell develops into a plasma cell can be divided into three phases: *maturation* in the bone marrow from stem cell to an immature B cell, *activation* of the B cell via an encounter with an antigen, and *differentiation* of the B cell into short-lived plasma cells, memory B cells, and long-lived plasma cells (Kindt et al., 2007a).

B-Cell Maturation in the Bone Marrow

The maturation process for B cells begins within the bone marrow as a hematopoietic stem cell progresses through several stages of development and culminates into an immature B cell (Caers, Van Valckenborgh, Menu, Van Camp, & Vanderkerken, 2008). It is important to note that in the beginning stages of differentiation the stem and progenitor cells do not express any immunoglobulin on the cell surface and only at the precursor and immature stage do membrane receptors

begin to emerge (Abbas et al., 2000). A B lymphocyte is unique in that it is the only cell that expresses an antibody on the cell surface membrane.

There are many cells and growth factors that promote the differentiation of B cells but a critical driving mechanism is the continuous rearrangements of the B cell's DNA, referred to as gene rearrangement (Dorshkind, 2000; Kindt et al., 2007c). These gene rearrangements drive the maturation process of the B cell and the evolution of surrogate precursors of antibodies from within the cytoplasm to fully expressed antibodies on the cell surface (Meffre, Casellas, & Nussenzweig, 2000). Figure 1 depicts each stage occurring in the bone marrow, the corresponding gene rearrangements, and the level of immunoglobulin expression that occurs at each stage of development. Additionally, changes in the expression of cell surface markers are noted for each stage of development and highlight the utility of flow cytometry in diagnosing B-cell malignancies.

B-Cell Activation

Immature B cells exit the bone marrow, and after entering into the periphery will only express IgM. The immature B cell will undergo further development to eventually coexpress both IgM and IgD (Meffre et al., 2000). During this transition the B cell may be referred to as a naive or transitional B cell (Billadeau et al., 1993). Upon coexpression of both IgM and IgD, the B cell is considered mature and is now capable of mounting a response to an antigen. Additionally, as these B cells are released into the periphery they will migrate to secondary lymphoid organs: the follicular zone in a lymph node or the marginal zone in the spleen (Kindt et al., 2007a). Secondary lymph organs provide the sites for B cells to encounter antigens. Mature B cells become activated when the antibody expressed on the cell surface binds with an antigen. Once activated, the B cell enters into the next phase of development known as differentiation.

B-Cell Differentiation

The activated B cell, or centroblast, will migrate into germinal centers in the lymph node or spleen and undergo rapid cell division (Kindt et al., 2007a). A myriad of complex interactions will occur between T cells, activated B cells, and follicular dendritic cells that will result in the selection and clonal expansion of the B cell capable of producing the specific antibody to eliminate the antigen (see Figure 1). During this active period *centroblasts* will undergo further gene rearrangement and antibody class switching, ultimately evolving into plasmablasts or memory B cells (Caers et al., 2008). Plasmablasts will undergo further differentiation into short-lived plasma cells that, along with the memory B cells, leave the germinal center and enter the periphery. A few plasmablasts will migrate to specific niches in the bone marrow that will induce further differentiation into long-lived plasma cells (Hallek et al., 1998). In healthy individuals, long-lived plasma cells only make up 0.25% of mononucleated cells in the bone marrow (BM) and function similar to a memory B cell, providing for a rapid response if there is repeated exposure to the same antigen.[1]

Immunoglobulin Structure

One of the clinical hallmarks of myeloma is the overproduction of a monoclonal protein. In contrast, healthy individuals produce about 3 g of antibodies every day by a variety of B lymphocytes, providing for polyclonal production of antibodies (Alberts et al., 2002). Immunoglobulins are Y-shaped proteins composed of basic structural units called chains (Kindt et al., 2007c). Each antibody contains two heavy chains and two light chains, of which each contains a constant and variable region as depicted in Figure 2. Protein chains are a sequence of amino acids; constant regions were identified when similar amino acid sequences were noted among numerous antibodies (Abbas et al, 2000). Light chains are noted to have two distinct types of pattern that are classified as either kappa or lambda in contrast to constant regions in heavy chains, which demonstrate five distinct patterns and are classified as IgG, IgM, IgA, IgE, or IgD. The heavy-chain portion determines the class or isotype of the immunoglobulin and each class may pair with either a

[1]The frequency of IgG myeloma is ~70% followed by IgA myeloma (20%–30%), with less then 1% of patients being diagnosed with IgM, IgE, or IgD myeloma. This may be partially explained by the differentiation pathway—note B cells express IgM and IgD at a pre–plasma cell stage and primarily secrete IgG and IgA when transformed to plasma cells. This is why myeloma is considered a mature B-cell malignancy, occurring at the latest stage of development (Munshi & Anderson, 2008; Billadeau et al., 1993; Hallek et al., 1998).

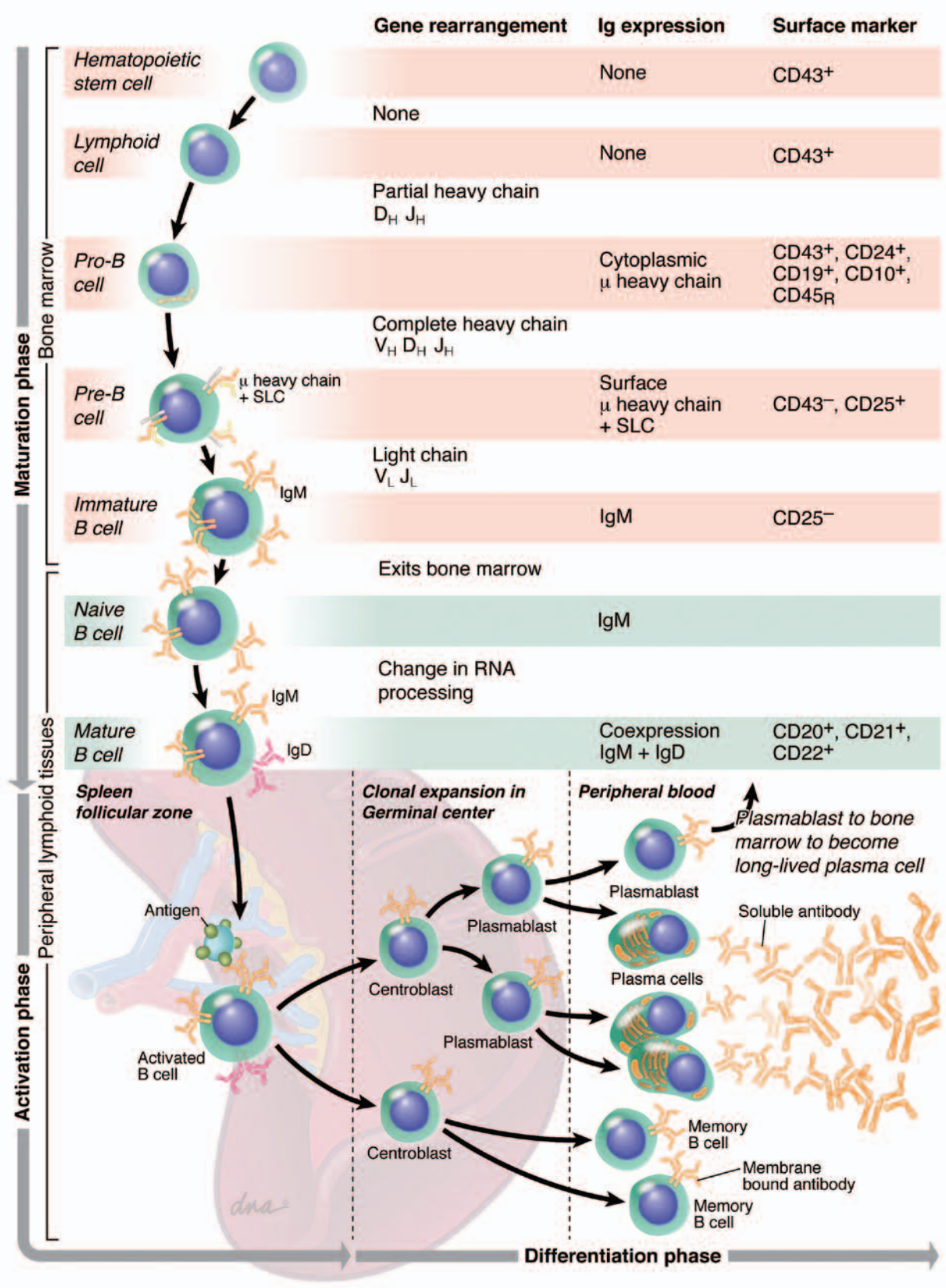

Figure 1. B-lymphocyte differentiation. D = diversity; J = joining; Pre-B cell = precursor B cell; Pro-B cell = progenitor B cell; SLC = surrogate light chain; μ heavy chain = Ig heavy chain membrane/cytoplasmic form; V = variable. (Abbas et al., 2000; Kindt, Goldsby, & Osborne, 2007a, 2007b, 2007c). Artwork by Alexandra and David Baker © 2010 DNA Illustrations, Inc.

kappa or lambda light chain.

The variable regions of heavy and light chains are located at the tip of the protein molecule and are the sites where antigens bind to the antibody. Although immunoglobulins may share a class via similarities in the constant region, each one is unique in the sequence of genes located on the variable region. It is this variability that allows for the creation of millions of different types of antibodies that can specifically identify a huge repertoire of different antigens (Doan, Melvold, Viselli, & Waltenbaugh, 2008).[2]

Scientists have been able to identify the genes responsible for coding the variable (V) and constant (C) regions of immunoglobulin structures (Abbas et al., 2000). As highlighted earlier, these regions undergo repetitive rearrangement throughout the lifespan of a B cell into a plasma cell. The sections of genes responsible for encoding heavy and light chains are known as gene segments. Lambda and kappa light-chain gene segments are located on chromosomes 22 and 2, respectively, with heavy-chain gene segments found on chromosome 14 (Doan et al., 2008). Light-chain multigene families contain V, J (joining), and C gene segments. VJ segments encode for the variable region of the light chain and C gene segments code for the constant region. Heavy-chain families contain V, D (diversity), J, and C gene segments with VDJ encoding the variable region and C encoding the constant region (see Figure 1 to note the gene segments rearranged per stage of B-cell development).

In summary, the pathway of B-cell differentiation into a plasma cell is a complex and multistep process that occurs within the bone marrow and secondary lymphoid organs. Malignant transformation can occur at any stage of cell development and explains the wide range of B-cell malignancies as well as plasma cell disorders. Specifically in myeloma, the heterogeneity of the disease is well understood when one considers the immense diversity among this class of protein molecules conferred via the variable regions in immunoglobulins. Although the cell origin of myeloma is still unknown, the use of DNA sequence analysis on MM cells demonstrates gene rearrangement patterns indicative of a postgerminal B cell, suggesting that the malignant transformation may evolve from plasmablasts or long-lived plasma cells (Caers et al., 2008; O'Connor et al., 2003; Chng, Glebov, Bergsagel, & Kuehl, 2007). The exact site of malignant transformation continues to be an area of discussion as some abnormal changes could possibly occur in the periphery followed by a homing of the malignant cell to the bone marrow, at which point the microenvironment will induce further malignant changes (O'Connor et al., 2003).

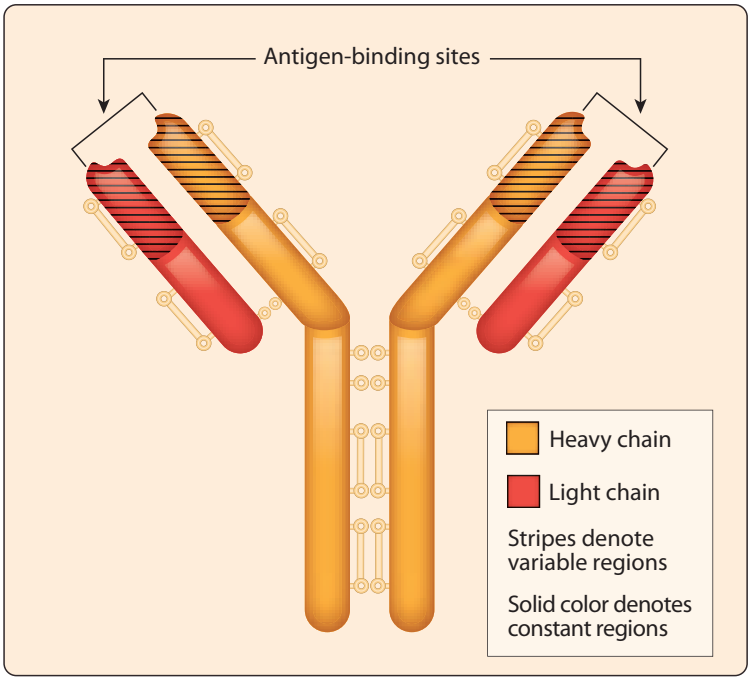

Figure 2. Immunoglobulin structure. Heavy chain depicted in beige; light chain depicted in red. Figure reprinted with the permssion of Celgene.

Anatomy of the Bone Marrow Microenvironment—The Niche

The bone marrow is organized into a complex network of submicroenvironments known as niches. Niches may be thought of as encapsulated chambers composed of cellular, noncellular, and liquid compartments that work in concert to achieve he-

[2]In a majority of myeloma cases the M protein produced will contain a heavy and a light chain. However, up to 30% will overproduce only the light-chain portion; this is known, appropriately, as light-chain myeloma. Understanding the molecular structure of immunoglobulins will guide APs in ordering tests for their myeloma patients. Light chains are too small to be detected by serum protein electrophoresis and necessitate the use of free light chain assays to measure the M protein (Durie, 2007; Doan et al., 2008).

Table 1. Bone Marrow Compartments

Cellular	Noncellular (extracellular matrix)	Liquid
Hematopoietic	*Structural proteins*	bFGF
Dendritic cells	Collagen	CD40
Erythrocytes	Elastin	HGF
Hematopoietic stem cells	*Protein polysaccharide complexes*	IGF-1
Lymphocytes	Proteoglycans	IL-6
Macrophages	*Adhesive glycoproteins*	IL-10
Megakaryocytes	Fibronectin	IL-11
Natural killer cells	Laminin	IL-15
Platelets		IL-21
Progenitor/precursor		MIP-1α
Nonhematopoietic		SDF-1α
Chondrocytes		TNFα
Fibroblasts		VEGF
Osteoblasts		Wnts
Osteoclasts		
Stromal cells		

Note. See Table 2 for abbreviations. Adapted, with permission, from Macmillan Publishers Ltd, "Bone marrow microenvironment and the identification of new targets for myeloma therapy," by K. Podar, D. Chauhan, and K. C. Anderson, 2009, *Leukemia, 23,* pp. 10–24.

matopoiesis: a finely tuned and highly regulated process (Podar, Richardson, Hideshima, Chauhan, & Anderson, 2007). The cellular compartment consists of nonhematopoietic cells such as fibroblasts, osteoclasts (OCs), and osteoblasts (OBs), as well as hematopoietic cells such as stem cells, progenitor cells, and immune function cells. These cells are suspended and nurtured in a mesh of insoluble proteins collectively known as the extracellular matrix (ECM) or the noncellular compartment.

There are three classes of molecules found in the ECM: structural proteins (collagens and elastins), protein-polysaccharide complexes (proteoglycans), and adhesive glycoproteins (fibronectins and laminins) (Podar et al., 2009). The ECM provides for numerous functions, including segregating tissues from one another, regulating intercellular communication, and serving as an anchorage site for cells and a depository for the liquid compartment. The liquid compartment consists of numerous proteins such as cytokines, growth factors, and other adhesion molecules that provide for the indirect communication between cells. These signaling molecules can target either the same cell (autocrine) or an adjacent cell (paracrine) and ultimately cause a change in cellular function (Kindt et al., 2007). There are hundreds of cytokines and they are organized into families of proteins that provide similar functions. For example, chemokines are a family of small cytokines that attract cells toward them and are thought to play a role in homing and angiogenesis (Caers et al., 2008). The compartments of the BMME are highlighted in Table 1.

Myeloma and the BMME

In MM, the malignant cell disrupts the normal balance within the microenvironment by forming synergistic relationships with key cellular and noncellular components as well as causing dysregulation of multiple cytokines (Kotla et al., 2009; Podar & Richardson, 2007). In the simplest analogy, the MM cell becomes the "commander" of the bone marrow microenvironment and as a result establishes a site that is permis-

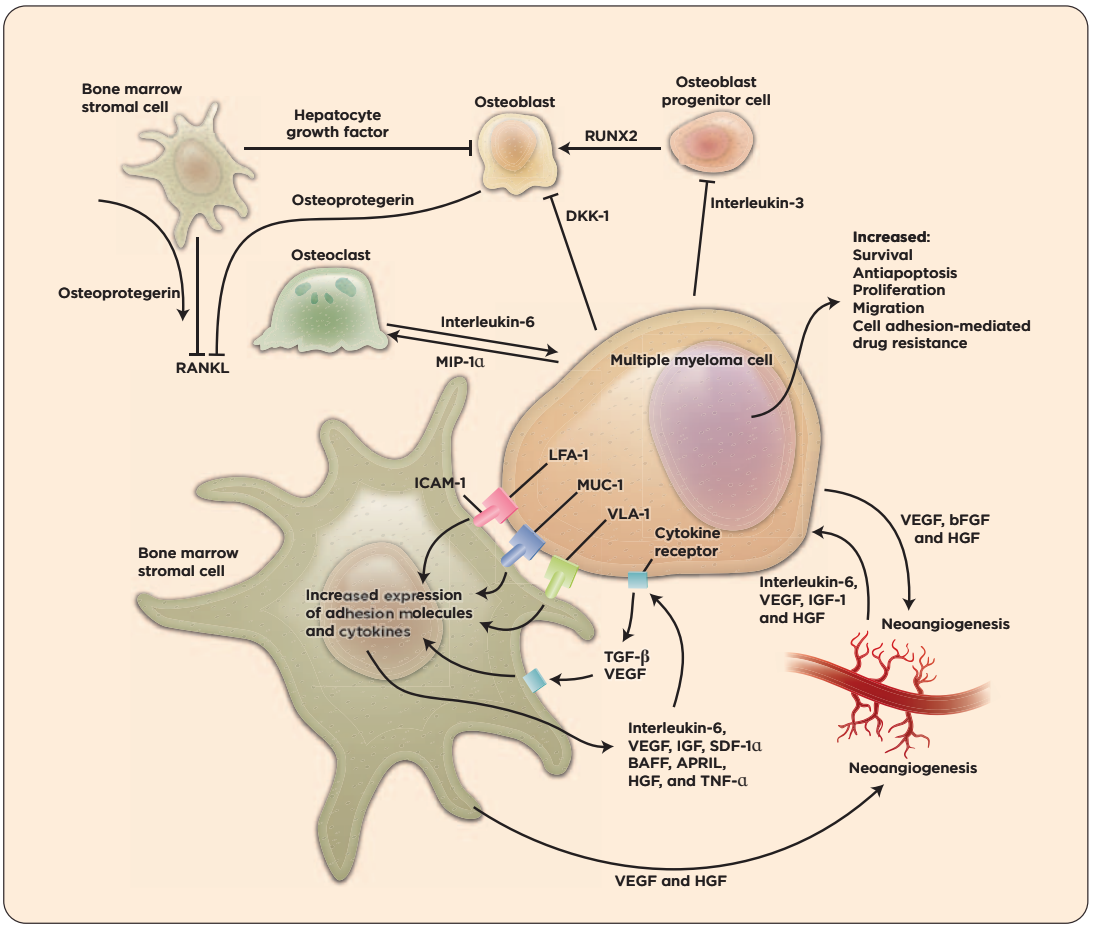

Figure 3. Cellular and signaling interactions between the multiple myeloma cell and bone marrow microenvironment. See Table 2 for abbreviations. Reprinted, with permission, from "Autologous hematopoietic stem-cell transplantation for multiple myeloma," by J. L. Harousseau and P. Moreau, 2009, *New England Journal of Medicine, 360,* pp. 2645–2654. Copyright © 2009 Massachusetts Medical Society. All rights reserved.

sive and supportive of tumor growth (J. Mikhael, personal communication, 2010). Multiple myeloma cells are able to exert their effect on the BMME by sending signals that will change the behavior of the cells and the ECM. Conversely, the BMME will also provide signals back to the MM cell; as this "crosstalk" continues to evolve, it forms a repetitive cycle that contributes to the promalignant state of the site (Corre et al., 2007). Although it is not all-inclusive, Figure 3 illustrates several of the important cellular and signaling interactions that occur between the MM cell and the BMME. In addition to knowing the key components that interact with the MM cell, a working knowledge of the molecular mechanisms of cellular communication is paramount to understanding the pathogenesis of multiple myeloma.

MOLECULAR MECHANISMS OF COMMUNICATION IN THE BMME

Cellular communication is achieved via the binding of transmembrane receptors that will trigger an intracellular signaling pathway (Caers et al., 2008). There are two forms of communication: (1) *direct,* when receptors bind to other receptors located on different cells or on ECM proteins, and (2) *indirect,* when liquid components such as cytokines and signaling molecules bind to receptors (Podar et al., 2009). Once triggered, signaling cascades mediate numerous molecular events within the target cell and result in a change in the cell's behavior. Molecular events that occur include cytoplasmic sequestration of transcription factors, changes in expression patterns of cell surface molecules, and secretion of growth and antiapoptotic factors (Hideshima & Raje, 2007). There are

Table 2. Molecules Active in the Myeloma Microenvironment

Cytokines
Angiopoietin-1 (Ang-1)
A proliferation-inducing ligand (APRIL)
B-cell activating factor (BAFF)
Hepatocyte growth factor (HGF)
Insulin-like growth factor-1 (IGF-1)
Interleukin-3 (IL-3)
Interleukin-6 (IL-6)
Transforming growth factor–beta (TGFβ)
Tumor necrosis factor–alpha (TNFα)
Angiogenic Proteins
Fibroblast growth factor–2 (FGF-2)
Vascular endothelial growth factor (VEGF)
Antiapoptotic Proteins
B-cell lymphoma–extra large (BCL–xL)
Bone-Remodeling Proteins
Dickkopf-1 (DKK1)
Osteoprotegerin (OPG)
Receptor activator of nuclear factor kappa B ligand (RANKL)
Runt-related transcription factor (RUNX-2)
Proteases
Matrix metalloproteinase-2 and -9 (MMP-2 and MMP-9)
Chemokines
Macrophage inflammatory protein-1 (MIP-1)
Stromal cell-derived factor-1 (SDF-1)
Signaling Pathways
Janus kinase–signal transducer and activator of transcription 3 (JAK2/Stat3)
Nuclear factor kappa B (NF-κB)
Phosphoinositide 3-kinase (PI3K/AKT)
Ras/mitogen-activated kinase/mitogen-activated protein kinase (Ras/MEK/MAPK)
Wingless type (Wnt)
Receptors/Adhesion Molecules
Integrin beta-1 (Integrin-β1)
Intercellular adhesion molecule-1 (ICAM-1)
Leukocyte function-associated antigen-1 (LFA-1)
Vascular cell-adhesion molecule (VCAM-1)
Syndecan-1
Tyrosine kinase receptors (FGFR1–FGFR4)
Very late activating antigen-4 (VLA-4)

Note. Based on information from "Importance of the bone marrow microenvironment in inducing the angiogenic response in multiple myeloma," by D. Ribatti, B. Nico, and A. Vacca, 2006, *Oncogene, 25,* pp. 4257–4266.

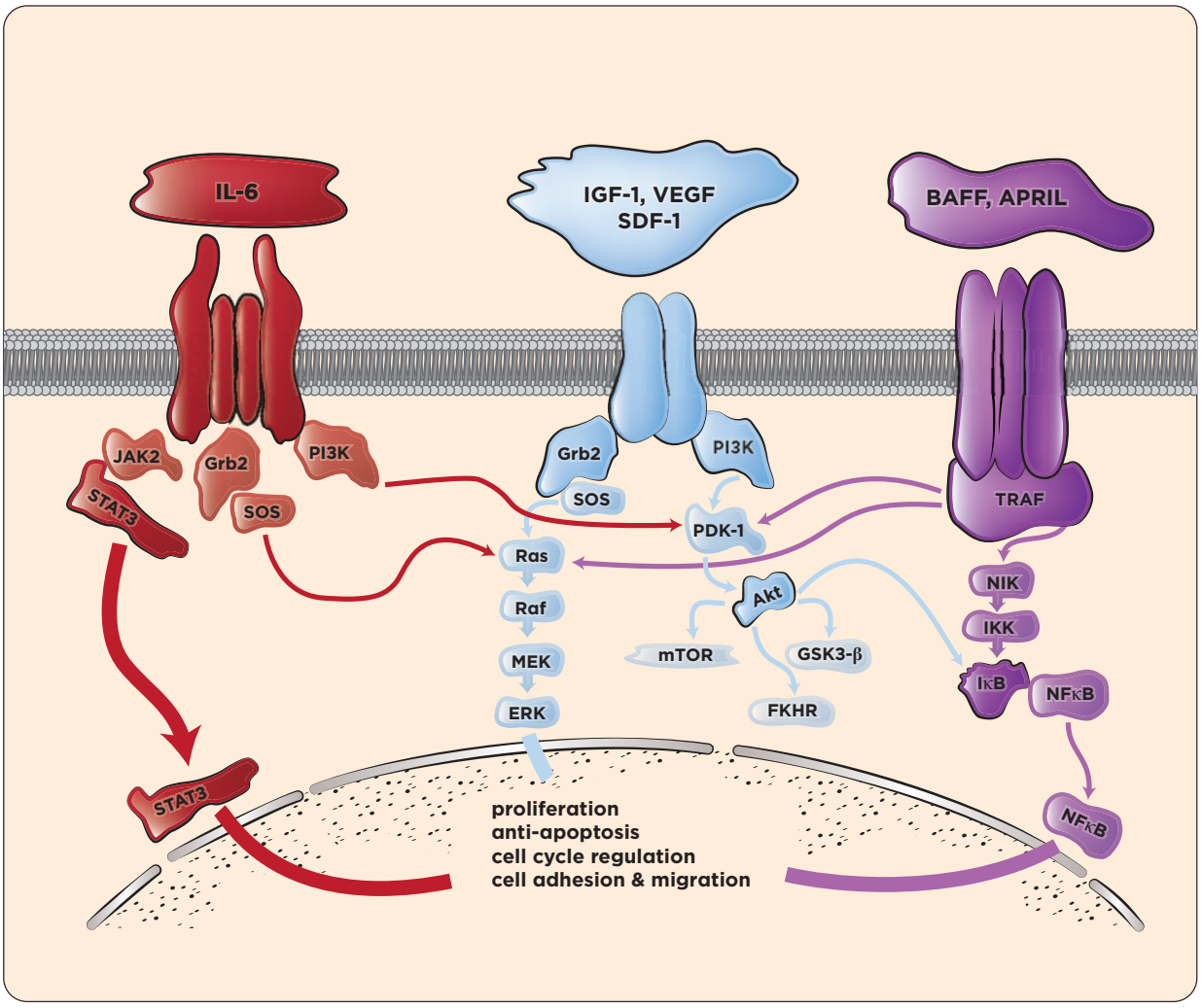

Figure 4. Interactions among signaling molecules, receptors, and signaling pathways. ERK = extracellular signal–regulated kinase; mTOR = mammalian target of rapamycin. See Table 2 for all other abbreviations. Reprinted, with permission, from "Unraveling the biology of multiple myeloma disease: Cancer stem cells, acquired intracellular changes and interactions with the surrounding micro-environment," by J. Caers, 2008, *Bulletin Cancer, 95*(3), pp. 303–313.

a large number of cell surface receptors, and they can be further categorized according to the cellular functions they affect. In the study of MM, a group called cell adhesion molecules (CAMs) is currently of particular importance. Cell adhesion molecules are receptors that provide stable cell-cell or cell-ECM contact (Kindt et al., 2007c). Integrins, selectins, and cadherins are all types of cell adhesion molecule receptors (Abbas et al., 2000). The expression pattern of cell surface receptors is important and will define how MM cells can communicate to other compartments of the BMME. Table 2 lists some of the key molecules, receptors, and signaling pathways that are known to be important in the pathogenesis of myeloma. Figure 4 is an illustration of how signaling molecules, receptors, and signaling pathways interact.[3]

Acquired Capabilities of MM: Hallmarks of Malignancy

Cancer cells have several unique capabilities that alter the malignant cell's physiology and work cohesively to support carcinogenesis (Hanahan & Weinberg, 2000). To date six capabilities

[3]Beta-2-microglobulin is a protein found in association with the major histocompatibility complex class I heavy chain on the surface of nearly all cells. It is overexpressed on myeloma cells and therefore serves as a rough correlate for tumor burden; it is recognized as a strong predictor of survival in patients with multiple myeloma (Podar et al., 2009; Hideshima & Raje, 2007).

or "hallmarks of cancer" have been proposed: (1) insensitivity to antigrowth factors, (2) tissue invasion and metastasis, (3) limitless replicative potential, (4) altered epigenetic control, (5) evasion of apoptosis, and (6) self-sufficiency to growth factors (i.e., malignant cells will reduce dependence on exogenous growth factors and generate their own; Hanahan & Weinberg, 2000). Recently, Caers et al. (2008) attributed the replicative potential of malignant cells to three of these capabilities: "growth signal autonomy, insensitivity to antigrowth signals, and resistance to apoptosis leads to an uncoupling of a cell's growth program from signals within its environment with acceleration of tumor growth and inhibition of tumor cell death" (Caers et al., 2008, p. 306). Similarly, MM cells will acquire these advantageous characteristics throughout all phases of the disease. During the initial stages, MM cells selectively home toward the BMME. Homing signals not only encourage migration to the microenvironment, but may also assist the MM cell in forming relationships with other cells and the ECM. Complex networks of growth factors, cell surface receptors, and signaling molecules will establish lines of communication between the MM cell and the BMME that will enable the MM cells to acquire hallmark capabilities critical to their survival (Hallek et al., 1998). As the disease progresses, tumor-induced angiogenesis, development of drug resistance, immunosuppression, and lytic lesion formation will also occur. Some of the key cellular/noncellular components, growth factors, receptors, and signaling pathways that contribute to these properties are discussed below.

GROWTH AND SURVIVAL

Initial tumor growth and survival for MM is primarily derived from the interaction between myeloma cells and bone marrow stromal cells (BMSCs). Tumor necrosis factor–alpha (TNFα) secreted by macrophages plays an important role in establishing the initial relationship between MM cells and BMSCs. TNFα primarily acts upon BMSCs causing a fivefold increase in the secretion of interleukin (IL)-6, a potent growth factor for MM cells (Corre et al., 2007; Costes et al., 1998). TNFα also induces overexpression of numerous integrin molecules on both MM cells and BMSCs, allowing for increased adhesion between these cells (Podar, Ghobrial, Hideshima, Chauhan, & Anderson, 2007; Caers et al., 2008). IL-6, probably the most important growth factor in myeloma, is predominantly produced in a paracrine fashion (Hallek et al., 1998). Multiple myeloma cells will express VLA-4 (an integrin), which will bind with VCAM-1 on BMSCs resulting in increased IL-6 secretion by the BMSCs (O'Connor et al., 2003). IL-6 also serves as a trigger for multiple signaling pathways critical to MM development, including Ras/MEK/MAPK, JAK2/Stat3, and PI3K/Akt (Podar et al., 2009).[4]

Ras/MEK/MAPK is induced indirectly by IL-6, IGF-1, and VEGF as well as directly via cell-to-cell contact through integrins (Downward, 2003). Activation of this signaling pathway contributes to myeloma cell proliferation and enhances drug resistance (Hideshima & Raje, 2007). The JAK2/Stat3 pathway, when activated, leads to myeloma cell survival by increasing the expression of BCL-xl and Mcl-1—two potent antiapoptotic proteins. Overexpression of these molecules on MM cells results in apoptotic defects that are associated with chemoresistance and aggressiveness of tumor cells (Caers et al., 2008). Mcl-1 is essential for the survival of human MM cells in vitro and is overexpressed in the tumor cells of patients with relapsed or poor prognosis MM (Hideshima & Raje, 2007). Similar to the JAK2 pathway, P13K/Akt is also activated by IL-6, IGF-1, and VEGF. Triggering of the P13K component of this pathway mediates MM cell growth where as Akt inhibits proapoptotic proteins and specifically allows for the IL-6 blocking of dexamethasone-induced apoptosis (Hideshima & Raje, 2007).

BAFF (B-cell activating factor) and APRIL (a proliferation and inducing ligand) are both members of the TNF family and are normally important for B-cell development and homeostasis (Caers et al., 2008). In multiple myeloma these protein molecules are overexpressed on MM cells and BMSCs and are considered to provide support for MM cell growth as well as adhesion to BMSCs by triggering the NF-kB, PI3K/Akt, and Ras/Raf/MAPK pathways. They contribute to tumor survival by enabling the cells to evade the apoptosis that would normally occur if they were deprived of IL-6 or exposed to dexamethasone (Podar et al., 2009).

[4]Corticosteroids (dexamethasone and prednisone) were one of the first class of medications noted to have an impact on myeloma. The antimyeloma effect is attributed to the anti–IL-6 properties of these drugs (Hideshima & Raje, 2007).

Another key cell adhesion molecule expressed on MM cells is B1 (CD29), which mediates MM cell adhesion to endothelial cells, fibroblasts, and the ECM. Adhesion to these cells critically enhances MM cell growth and survival as well as protects against drug-induced apoptosis (Podar & Ghobrial et al., 2007). Specifically, VEGF will cause MM cells to migrate and adhere to fibronectin, triggering a multitude of signaling via various integrin complexes (Hideshima & Raje, 2007). These interactions directly protect the MM cells from DNA damaging drugs such as anthracyclines and alkylating agents and are collectively referred to as cell adhesion–mediated drug resistance (Shain et al., 2009).

TUMOR INVASION AND MIGRATION

Tumor invasion and migration are properties that are increased in MM cells and are important steps in the spreading of disease. Interestingly, MM cells rarely metastasize to other organs but rather circulate to new niches, allowing for tumor cell expansion (Billadeau et al., 1993). Transport between niches is achieved when MM cells are able to attach to and degrade the basement membrane of the ECM. The basement membrane, composed of collagen and glycoproteins, primarily serves to provide a barrier between the niches (Broek et al., 2001). Certain components of the ECM have been shown to stimulate migration of MM cells as well as cause adhesion and upregulation of substances that will breakdown the basement membrane. For example, MM cells will begin to migrate toward fibronectin in response to vascular endothelial growth factor (VEGF) secreted by endothelial cells and BMSCs. VEGF binds to VEGF receptor 1 on MM cells and migration is thought to be induced via the PI3K pathway (Caers et al., 2008). Adhesion of MM cells to fibronectin will increase the production and release of matrix metalloprotein-2 (MMP-2) and MMP-9. Matrix metalloproteins are a family of proteolytic enzymes that contribute to the invasion of tumor cells through their ability to penetrate and infiltrate the stromal matrix (Caers et al., 2008). Additionally, MMPs are also capable of activating different growth factors (via the ECM), suppressing tumor cell apoptosis, and releasing angiogenic factors.

ANGIOGENESIS

Angiogenesis, the formation of new blood vessels from existing vasculature, has long been recognized as a key characteristic of tumor cell growth and spread (Folkman, 2002). It is a multistep process facilitated by the migration and growth of endothelial cells to build new vessels. Neovascularization not only provides the needed nourishment for malignant cell proliferation, but the growth pattern may also provide additional advantages to the MM cells. The growth pattern of tumor-induced angiogenesis is considered chaotic and is referred to as mosaic (Podar et al., 2009). This abnormal structure leads to aberrant blood flow and vessel leakiness, possibly contributing to decreased drug delivery to areas of tumor growth (Podar et al., 2009). Clinically, bone marrow angiogenesis is a hallmark of MM progression, with higher microvessel density in the bone marrow being correlated to the transition from monoclonal gammopathy of undetermined significance (MGUS) to MM, or from remission to relapsed disease, and is an indicator of poor prognosis (Vacca & Ribatti, 2006).

Angiogenic switch is a term used to define the change in tumor cell growth from avascular to vascular (Folkman, 2002). In myeloma, angiogenesis is primarily "switched on" by the activities of MM cells and BMSCs. Both cells promote vessel formation through the secretion of proangiogenic growth factors while simultaneously inhibiting antiangiogenic factors. VEGF, a very potent angiogenic cytokine produced by MM cells and BMSCs, will bind to VEGF receptors on endothelial cells triggering a phosphorylation of the tyrosine kinase pathway (Podar et al., 2001). Additionally, VEGF is known to recruit endothelial progenitor cells to vascular sites. Matrix metalloproteins also play an important role in angiogenesis via the ability to remodel ECM and encourage migration of EC cells.

Fibroblast growth factor receptor (FGF), another potent angiogenic cytokine, is primarily produced by MM cells and targets BMSCs. Bone marrow stromal cells express a family of four distinct tyrosine kinase receptors (FGFR1–FGFR4) that have a high affinity for binding with FGF, which when activated increases IL-6 production (Leleu & Anderson, 2007). IL-6 in turn will stimulate the MM cells to overexpress and secrete more FGF triggering—not only angiogenesis but MM cell growth and survival as well.[5]

LYTIC LESIONS

Lytic lesions are another hallmark of multiple myeloma and generally occur in areas near MM cells, suggesting that this process is not only mediated by cytokines but also requires cell-to-cell contact (Yaccoby, 2010b). Normal skeletal structure is maintained by an intricate balance of osteoblasts (bone formation) with osteclasts (bone resorption). In contrast to solid tumor malignancies, which invade the bone, MM cells disrupt the bone remodeling process by simultaneously inducing osteoclast activation coupled with osteoblast inhibition (Edwards, Zhuang & Mundy, 2008; Mitsiades, Mitsiades, Munshi, Richardson, & Anderson, 2006). It may also be thought of as a form of accelerated osteoporosis that ultimately leads to bone destruction. In myeloma, lytic lesion formation is mitigated via a complex array of relationships between MM cells, BMSCs, osteoblasts, and osteoclasts that induce key ligands and signaling pathways.

Osteoclasts can be stimulated by a variety of growth factors such as RANKL (receptor activator for nuclear factor κ B ligand), VEGF, TNFα, and IL-3 that are collectively referred to as the osteosclast activating factors (Mitsiades et al., 2006). One of the most potent of these is RANKL, a surface-bound molecule that is expressed on many cells, including endothelial cells and OBs, but in myeloma is predominantly overexpressed by the BMSCs (Roodman, 2010). It is a part of the TNF gene family and can be bound by cell-cell adhesion or indirectly through various other cytokines (Edwards et al., 2008). Upregulation of RANKL on the surface of BMSCs is primarily induced by the adhesion of MM cells to BMSC. Bone marrow stromal cells will then bind to RANK receptors on OCs and OC precursors, triggering differentiation and growth of mature OCs, OC activation, and inhibition of OC apoptosis signals (Roodman, 2010). Other cytokines that induce osteoclastogenesis include TNFα, MIP1α, VEGF, and IL-3, which indirectly enhances the effect of RANKL and MIP1α on OC.

Changes in the BMME induced by myeloma not only promote unchecked osteclastogenesis but deactivate the formation of new OBs, resulting in inhibition of bone formation. The Wnt pathway plays an important role in the growth, development, and functioning of osteoblasts (Heath et al., 2009). There are several soluble proteins that inhibit the Wnt pathway, including DKK1 (dickkopf-1), which is produced in excess by MM cells (Yaccoby, 2010a). Excess amounts of DKK1 will inhibit OB formation and cause changes in the molecular expression of mature OB by increasing RANKL and decreasing osteoprotegerin (OPG; Yaccoby, 2010a). Osteoprotegerin is a RANKL inhibitor produced by OBs and BMSCs that normally interferes with osteoclast activation. Osteoprotegerin generally counteracts the stimulatory effect of RANKL by acting as a decoy binding molecule for the RANK receptors on OCs. In healthy individuals, maintaining the correct ratio of RANKL to OPG is one of the safeguard mechanisms against bone destruction by determining the rate of osteoclast formation and activity (Roodman, 2010). Multiple myeloma adhesion to BMSCs not only increases RANKL expression but also leads to decreased production of OPG on BMSCs, allowing for increased amounts of RANKL binding to OCs. In addition, RUNX2, a transcription factor critical for osteoblast differentiation, is blocked by MM cells in osteoblast progenitors through direct VLA-4/VCAM-1–mediated contact and/or IL-7 secretion (Podar & Richardson et al., 2007). Ultimately, the culmination of all of these interactions tips the balance of bone remodeling to bone destruction and the formation of lytic lesions.

IMMUNOSUPPRESSION/IMMUNE MODULATION

Immunosuppression in myeloma patients is not only a major contributing factor to morbidity, with infections being a primary cause of death, but may also contribute to the growth and proliferation of MM (Blade & Rosinol, 2007). The most direct effect myeloma has on the immune system is the overproduction of monoclonal proteins incapable of providing any immune defense coupled with the decreased production of polyclonal healthy immunoglobulins (Terpos, Cibeira, Blade, & Ludwig, 2009). Additionally, changes in the BMME also inhibit the normal

[5]Immunomodulatory drugs and proteasome inhibitors are considered novel agents and have significantly contributed to the treatment of patients. Both are thought to have antiangiogenic properties that contribute to the impact these agents have on multiple myeloma (Podar et al., 2009).

balance of hematopoiesis. As highlighted in the previous paragraph, the MM environment has an overproduction of DKK1, a Wnt pathway inhibitor, leading to decreased OB activity (Hideshima & Raje, 2007). The lack of new bone formation affects the ability of the BMME to support the differentiation of hematopoietic cells to maturation, thereby indirectly causing anemia and immunosuppression (Podar et al., 2007). Additionally, IL-6, TNF, and IL-1 may contribute to the inhibition of new blood formation (Podar & Richardson et al., 2007).

The ability to prevent cancer via the immune system may be accomplished via several avenues provided by the cellular (macrophages, dendritic cells, and lymphocytes) and humoral (cytokines and antibodies) components (Kotla et al., 2009). In healthy individuals both components work in concert to identify and eliminate any abnormal cells with a propensity for malignant transformation, as well as alter the microenvironment to be hostile towards malignant growth (Kotla et al., 2009). Changes in the BMME caused by MM may decrease T-cell, dendritic cell, and natural killer (NK) cell formation. As a result, MM cells are capable of reducing the surveillance provided by these cells and escape elimination via these mechanisms. Additionally, other hypotheses include lack of immunogenicity of tumors and the expression of ligands that inhibit NK cells (Caers et al., 2008).

Conclusions

In summary, the development of multiple myeloma can be viewed as a complex multistep process that involves genetic changes in the malignant plasma cell, as well as the supportive conditions of the BMME that stimulate MM cell growth, survival, migration, and drug resistance. Understanding the pathogenesis of MM has led to significant advances in treatment for these patients that have positively impacted overall survival and quality of life (Alsina & Richardson, 2007). It is imperative that the advanced practitioner have an in-depth and current understanding of this multistep process in order to prescribe appropriate therapies, manage adverse events, and monitor for disease response. Changes in the BMME not only contribute to the pathogenesis of multiple myeloma but contribute greatly to the complications of this disease.

DISCLOSURES

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