Immunotherapy for Pediatric Malignancies

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Chapter 1
Introduction to Pediatric Cancer Immunotherapy

Aurélien Marabelle and Claudia Rossig

Abstract Cancer immunotherapy comes of age for adult malignancies. Immune targeted antibodies aiming at disrupting immunosuppressive pathways such as the checkpoints PD-1/PD-L1 and CTLA-4/B7 are providing durable responses and overall survival benefits in multiple relapsing/refractory adult cancer types. Novel immunotherapies such as oncolytic viruses and adoptive CAR-T cells are also becoming approved immune therapies and revolutionize the world of drug development. These therapeutic innovations are currently fostering an unprecedented research effort in adult tumor immunology. Pediatric cancers have major histological, biological and developmental differences with adult cancers. Although the fundamental immunological rules remain the same between adults and children, the limited data currently available suggest that the immune cells and the immunosuppressive pathways that are at stake in pediatric cancers might be different than the ones acting in adult cancers. Clinical results of passive immunotherapy with tumor targeting antibodies, cytokines, bispecific T-cell engaging antibodies and CAR-T cells have recently demonstrated that pediatric cancers can be treated with immunotherapy. However, the benefits of these novel treatments are limited to a small fraction of pediatric cancers. Fundamental and translational research efforts are currently eagerly needed to better decipher what drives the immune surveillance and editing of pediatric cancers.

Keywords Pediatric tumors • Pediatric cancer • Immunotherapy • Immune system • Immune cells

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1.1 Introduction

During their evolution over the last 3 billion years, multicellular organisms have developed tissues and organs with refined specificities to allow better survival and interbreeding. Among the subsets of tissues which compose a vertebrate living organism, the immune system can be defined as the subsets of cells that are produced by the hematopoietic stem cells in the bone marrow but do not belong to the red blood cell and platelet lineages. These so called "white blood cells" or leucocytes are present throughout the body, either staying in tissues as resident cells since the early embryogenesis, or circulating through the tissues, blood vessels and lymphatic vessels of the body. They can directly contribute to the structure of specific organs of the body known as the primary and secondary lymphoid organs. Primary lymphoid organs include the bone marrow and the thymus where immune cells (lymphocytes for the thymus) are formed and mature. Secondary lymphoid organs include structures such as lymph nodes, tonsils, spleen, Peyer's patches and mucosa associated lymphoid tissue (MALT). These white blood cells, their protein products (cytokines, chemokines, antibodies), and their related organs are key elements of mammalians natural defenses against pathogens (virus, fungus, bacteria).

1.2 Overview of the Components of the Immune System

Immune cells can be divided in two subsets of cells: the innate immune cells and the adaptive immune cells (Fig. 1.1). Innate immune cells are granulocytes (neutrophils, basophils and eosinophils), monocytes/macrophages, mast cells and dendritic cells. They can react fast against pathogens in a stereotypic, pathogen non-specific manner and are devoid of memory features. Adaptive immune cells are B-cells and T-cells. These lymphocytes react more slowly than innate immune cells. They have memory features which allow them to react in a pathogen specific manner, and to increase this reaction over time. Some immune cells such as γδ T-cells and NK-T cells share some common features of both the innate and adaptive immune system as they can respond in an antigen specific and non-specific manner. All these immune cells act in coordination with each other over time and at the different sites of the body in order to maintain the homeostasis of the host. Communications between immune cells and other cellular components of the body is performed through cell-cell interactions, cytokines and chemokines. Detailed aspects of the composition and function of the immune system have been extensively reviewed in the literature, notably in the context of cancer [1].

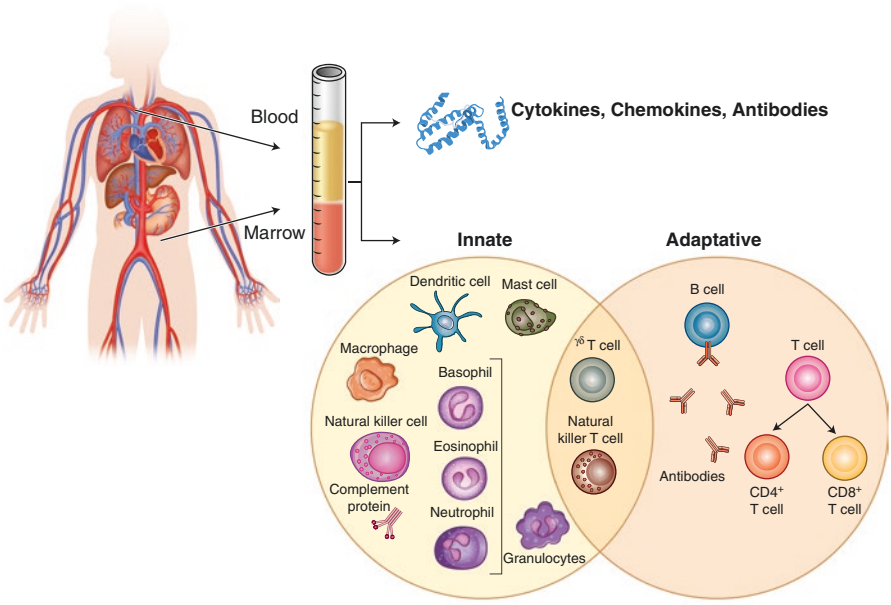

Fig. 1.1 Components of the immune system. The main effectors of the immune system have been described in the blood and bone marrow although specific tissue resident immune cells are not present in these compartment (e.g. some subsets of gamma deltaT-cells). Innate immune cells have rapid, stereotypic responses to dangers signals such as pathogens but are devoided of memory features. Alternatively, it takes a couple of weeks to the adaptive immune cells to generate a novel antigen-specific response, but its memory features provides more rapid and potent responses upon subsequent exposures

1.3 Role of the Immune System in Cancer Biology

1.3.1 Tumor Infiltrating Immune Cells and Immune-Editing

Besides cancer cells and stromal cells, the tumor micro-environment can be infiltrated by subsets of immune cells. Some of these immune cells can contribute to the anti-tumor immune response against cancer cells. These effector cells can be cytotoxic CD8+ T-cells, type 1T-helper cells (so called "Th1"), type 1 macrophages (so-called "M1"), B-cells (including differentiated, antibody producing, plasmocytes), natural killer cells (NK cells), NKT-cells, and γδ T-cells. Our understanding of cancer biology has evolved over the last 15 years thanks to the description of subsets of immune cells which protect cancer cells from anti-tumor "auto-reactive" immune cells. Indeed, because cancer cells "belong to the immunological "self", they can evade the immune system by using pathways and effectors that generate immune tolerance. Tolerogenic immune effectors are regulatory FOXP3-positive CD4+

T-cells (Tregs), type 2 macrophages (so-called "M2"), and other types of more undifferentiated myeloid cells also called myeloid derived suppressor cells (or "MDSC"). The balance between immune rejection and immune tolerance of cancer cells, and the subsequent Darwinian pressure of selection of the fittest sub-clones of cancer cells over time has been coined with the concept of tumor "immuno-editing" [2]. Pediatric tumors typically have only sparse infiltrates of lymphocytes [3], but CD8+ T cells capable of effector memory responses were found e.g. in neuroblastomas [4].

1.3.1.1 Tumor Antigens and Immunogenicity of Pediatric Tumors

Although tumor cells are immunologically "self", they can differ from healthy cells by the aberrant expression of molecules that can be recognized by the immune system (Fig. 1.2). On the other hand, they can secrete molecules or express ligands which can hamper immune cell functions.

1.3.1.2 Tumor-Specific Antigens of Pediatric Tumors

Somatic point mutations in the cancer cell DNA can lead to the expression of aberrant proteins. Peptides from these proteins can behave as neo-antigens when they become presented to T-cells via MHC molecules. Such neo-epitopes are

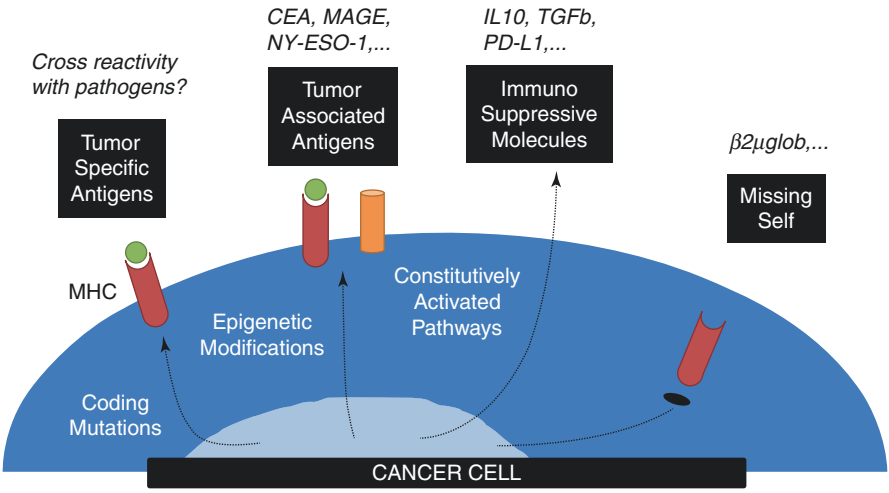

Fig. 1.2 Impact of genomic and epigenetic abnormalities on cancer cells immunogenicity. Cancer cells are "self" cells and can therefore use many physiological pathways to prevent an "auto" immune reaction (e.g. PD-L1 upregulation). However, the multiple genomic alterations happening in the cancer cell genome and the epigenetic changes have an impact on the overall immunogenicity of tumors. Some alterations can increase the cancer cell immunogenicity (e.g. tumor specific antigens presented by the MHC molecules upon somatic point mutations in the cancer cell genome). Others can dampen the recognition of cancer cells by the immune system (e.g. mutations in the beta2-microglobulin preventing functional presentation of MHC-I molecules)

tumor-specific antigens (TSA) and can generate tumor-specific T-cell responses. This phenomenon has been recently well described, and seems to play a significant role in the response to checkpoint blockade (CTLA-4, PD-1) immunotherapy in some adult cancers but also in biallelic mismatch repair deficiency hypermutant pediatric glioblastoma [5–8]. However, we do not know if it plays a significant role in the immunogenicity of other pediatric cancers. Pediatric cancers often carry chromosome rearrangements [9, 10], but generally have a low frequency of somatic point mutations [11–13]. Still, in some subsets of patients, notably of poor prognosis, the mutation rate can be higher. Indeed, it has been recently demonstrated that high-risk neuroblastomas have a higher level of somatic point mutations than neuroblastomas with a good prognosis [14]. Specifically, the neuroblastoma genome can undergo chromothripsis, a phenomenon where some areas of a given genome can undergo thousands of chromosome rearrangements in limited regions of some chromosomes [14]. Besides somatic point mutations, the analysis of pediatric tumor genomes has also revealed that they have frequent chromosome rearrangements [9, 10]. These chromosome rearrangements could in theory generate truncated or translocated abnormal proteins which could become TSA. This hypothesis remains to be explored.

1.3.1.3 Tumor-Associated Antigens of Pediatric Tumors

Besides somatic genome aberrations, cancer cells can undergo epigenetic modifications which can result to the aberrant expression of some molecules. For instance, cancer cells can express high levels of proteins that are usually only expressed during embryonic development or in limited subsets of cells related to germ cells. These so called "carcino-embryonic" or "cancer-testis" antigens, such as NY-ESO-1, CEA, MAGE, and many others (see [15] for review) can be highly expressed on cancer cells, either by membrane expression of the full length protein (with possible alternate splicing), and/or via MHC presentation of peptides. T-cell or B-cell (antibody) specific responses to these TAA have been described in detail in adult cancers over the last 20 years. Interestingly, IgG antibodies against NY-ESO-1 as well as CD4/CD8 T-cell specific responses to HLA-A2-restricted peptide NY-ESO-$_{1157–167}$ were found in children with NY-ESO-1 positive neuroblastoma [16]. Also, immunization with an autologous interleukin-2 gene transduced neuroblastoma tumor cell vaccine has been shown to generate specific antibody responses against neuroblastoma cells [17].

Epigenetic changes in cancer cells can also end up in modifications of ganglioside expression. Gangliosides are sialic-acid-containing glycosphingolipids expressed on all vertebrate plasma membrane cells. Human healthy tissues usually do not express glycolylneuraminic acid containing gangliosides, but this molecule is expressed in tumors and in human fetal tissues [18]. Therefore, gangliosides are another type of onco-fetal TAA. Reminiscent of their neuroectodermal tissue origin, neuroblastomas express the ganglioside GD2 at high density. GD2 can also be overexpressed in Ewing sarcomas [19–21]. GD2 expression in neuroblastoma cells was suggested to contribute to tumor immune escape by negatively affecting the differentiation and capacity of dendritic cells to prime the proliferation of T-cells [22].

Anti-GD2 antibody therapy has been developed in the clinic and is becoming part of the standard of care of high-risk neuroblastoma [23–25]. More recently, GD2 is evaluated as an immune target also of redirected T cells (see Chap. 10).

Genetic and epigenetic changes in cancer cells can also result in the aberrant expression of intra-cellular proteins which can become TSA while being presented through the physiological MHC-I route. For instance, genomic alterations such as p53 inactivation can result in the upregulation of an intracytoplamic anti-apoptotic molecule called survivin. Interestingly, survivin-specific CD8+ T-cells have been detected in the blood of children with high risk neuroblastoma [26]. However, very few tumor infiltrating T-cells were found in the same patients, suggesting that immune cell infiltration into pediatric tumors may be a critical limitation to effective anticancer immune responses [26].

1.3.1.4 Immune Tolerance of Pediatric Cancer Cells

MHC Expression

Besides TSA and TAA, cancer cells can express molecules with immune-inhibitory function which contribute to their overall low immunogenicity. First, the downregulation or absence of expression of MHC-I molecules has been a classical mechanism of immune escape by preventing cancer cells to be recognized by CD8+ cytotoxic T-cells. Low or no MHC-I expression has been widely described in pediatric cancers [27]. However, downregulation of MHC-I is often reversible, and inflammatory conditions such as exposure to interferon-γ can upregulate MHC-I in most pediatric cancer cell lines [27, 28]. Sometimes, the absence of expression of MHC-I is a consequence of mutations in the beta-2 microglobulin, a protein which is part of the MHC-I complex. For instance, this has been recently described in about 70% of Hodgkin lymphomas [29]. The absence of MHC-I expression should in theory activate NK cells ("missing self" theory). Indeed, in neuroblastoma, where MHC-I molecules are often not expressed, NK cells were suggested to play a significant role in immune surveillance. One example is the recent finding that expression of distinct isoforms of the NK receptor NKp30, which can functionally interact with B7-H6 present in the serum of the patients in its soluble form and at the surface of tumor cells, is associated with survival in high-risk neuroblastoma patients [30].

Cytokines and Chemokines Expression

Cancer cells can further secrete cytokines either in an autocrine or paracrine manner which create a pro-tumoral inflammatory micro-environment. For instance, interleukin-6 (IL-6) has been found to be expressed by glioblastoma and neuroblastoma cells [31] but also by stromal cells in metastatic niches such as the bone marrow [32, 33]. IL-6 receptor (IL-6R) can also be expressed by neuroblastoma cells, and IL-6 from either cancer cells or metastatic bone-marrow on IL-6R positive

neuroblastoma cells can sustain their proliferation and prevent them from chemotherapy (etoposide)-induced apoptosis [33]. Also, IL-6 acts on myeloid derived osteoclast cells which can contribute to the development of metastatic bone marrow sites [33]. Accordingly, the circulating blood levels of IL-6 have been shown to be significantly higher in high-risk neuroblastoma [34], and the single nucleotide polymorphism rs1800795 in the promoter of the IL-6 gene (also known as the as the IL-6 "174" polymorphism) has been shown to have a prognostic value both in event-free and overall survival in children with high-risk neuroblastoma [35]. Also, interleukin-8 seem to play a role in neuroblastoma as both IL-8 and its receptor can be expressed on cancer cells [36]. Interestingly, treatment of neuroblastoma cells by retinoic acid (which is part of the standard of care of high risk neuroblastoma) stimulates IL-8 secretion by neuroblastoma cells and promote neutrophil and lymphocyte chemotaxis [37]. Both G-CSF and its receptor have been shown to be expressed by Ewing tumors, and osteosarcoma, and G-CSF has been shown to support Ewing xenograft tumor growth through both angiogenesis and leukocyte recruitment into tumors [38, 39]. However this data has been generated in immunocompromised xenograft models and might not be physiological. Subsequent concerns that G-CSF administration to promote granulocyte recovery post chemotherapy may be unsafe in Ewing sarcoma patients have not been substantiated, arguing against a relevant role of this pathway and GCSF remains part of the supportive care of Ewing sarcoma [40].

Chemokines can be critical for the infiltration of immune cells into the tumor microenvironment. In Ewing sarcoma, chemokine and chemokine receptor profiling revealed an association between an inflammatory immune microenvironment with infiltration by CD8+ T cells [41]. Genomic changes occurring in cancer cells can affect expression of chemokines. E.g., the oncogene MYCN, a hallmark of high-risk neuroblastoma, has been shown to repress the expression of CCL2 by neuroblastoma cells, a chemokine that can attract immune effector cells [42].

1.3.1.5 Immunosuppressive Pathways

Immunosuppressive ligands can be expressed on cancer cells. These so-called "immune checkpoints" can interact specifically with molecules expressed by immune cells and block their activation, induce tolerance and exhaustion. Programmed-death ligand-1 (PD-L1) is the most extensively studied immune checkpoint molecules in adult cancers. It interacts with the co-inhibitory receptor PD-1 which is expressed on lymphocytes. PD-L1 expression was also found in pediatric cancers such as neuroblastoma, nephroblastoma (Wilms tumor) and osteosarcoma [43–46]. Another potential tolerogenic immune checkpoint called B7-H3, and its isoform 4Ig6B7-H3, have been shown to be expressed in osteosarcoma and neuroblastoma, respectively [47, 48].

Tryptophane is a critical amino acid for the metabolism of immune cells, notably T-cells. The enzyme indoleamine 2,3-dioxygenase (usually called IDO) depletes tryptophan in the tumor micro-environment, and IDO expression has been

described as a key immunosuppressive pathway in many adult cancer types, notably under interferon-γ exposure. IDO has been shown to be expressed by osteosarcoma cell lines exposed to IL-12 and IL-18, suggesting a possible role in that pediatric cancer [49].

1.4 Conclusion

Overall, although the level of somatic point mutations remains low in pediatric cancer cell genomes, the cells can be immunogenic by other genomic and epigenetic alterations. Future research will have to identify the most relevant immune escape mechanisms in the biology of pediatric cancers to allow for effective intervention by immunotherapy. The subsequent chapters of this book will detail the immune contexture of pediatric cancers, the prognostic role of the different immune subsets and how they differ from adult cancers. Also, this book will provide a comprehensive overview of the various immunotherapy strategies under current development that aim to exploit the immune system to treat pediatric cancers.

References

1. Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer. 2004;4:11–22. doi:10.1038/nrc1252.
2. Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. 2011;331:1565–70. doi:10.1126/science.1203486.
3. Vakkila J, Jaffe R, Michelow M, Lotze MT. Pediatric cancers are infiltrated predominantly by macrophages and contain a paucity of dendritic cells: a major nosologic difference with adult tumors. Clin Cancer Res. 2006;12:2049–54. doi:10.1158/1078-0432.CCR-05-1824.
4. Carlson L, De Geer A, Sveinbjørnsson B, Orrego A, Martinsson T, Kogner P, et al. The microenvironment of human neuroblastoma supports the activation of tumor-associated T lymphocytes. Oncoimmunology. 2013;2:e23618. doi:10.4161/onci.23618.
5. Bouffet E, Larouche V, Campbell BB, Merico D, de Borja R, Aronson M, et al. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. J Clin Oncol. 2016;34(19):2206–11. doi:10.1200/JCO.2016.66.6552.
6. Boussiotis VA. Somatic mutations and immunotherapy outcome with CTLA-4 blockade in melanoma. N Engl J Med. 2014;371:2230–2. doi:10.1056/NEJMe1413061.
7. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015;348(6230):124–8. doi:10.1126/science.aaa1348.
8. McGranahan N, Furness AJS, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science. 2016;351:1463–9. doi:10.1126/science.aaf1490.
9. Janoueix-Lerosey I, Schleiermacher G, Michels E, Mosseri V, Ribeiro A, Lequin D, et al. Overall genomic pattern is a predictor of outcome in neuroblastoma. J Clin Oncol. 2009;27:1026–33. doi:10.1200/JCO.2008.16.0630.

10. Schleiermacher G, Janoueix-Lerosey I, Ribeiro A, Klijanienko J, Couturier J, Pierron G, et al. Accumulation of segmental alterations determines progression in neuroblastoma. J Clin Oncol. 2010;28:3122–30. doi:10.1200/JCO.2009.26.7955.
11. Lee R, Stewart C, Carter S. A remarkably simple genome underlies highly malignant pediatric rhabdoid cancers. J Clin Invest. 2012;122:2983–8. doi:10.1172/JCI64400DS1.
12. Downing JR, Wilson RK, Zhang J, Mardis ER, Pui C-H, Ding L, et al. The pediatric cancer genome project. Nat Genet. 2012;44:619–22. doi:10.1038/ng.2287.
13. Pugh TJ, Morozova O, Attiyeh EF, Asgharzadeh S, Wei JS, Auclair D, et al. The genetic landscape of high-risk neuroblastoma. Nat Genet. 2013;45:279–84. doi:10.1038/ng.2529.
14. Molenaar JJ, Koster J, Zwijnenburg DA, van Sluis P, Valentijn LJ, van der Ploeg I, et al. Sequencing of neuroblastoma identifies chromothripsis and defects in neuritogenesis genes. Nature. 2012;483:589–93. doi:10.1038/nature10910.
15. Gnjatic S, Nishikawa H, Jungbluth AA, Güre AO, Ritter G, Jäger E, et al. NY-ESO-1: review of an immunogenic tumor antigen. Adv Cancer Res. 2006;95:1–30. doi:10.1016/S0065-230X(06)95001-5.
16. Rodolfo M, Luksch R, Stockert E, Chen YT, Collini P, Ranzani T, et al. Antigen-specific immunity in neuroblastoma patients: antibody and T-cell recognition of NY-ESO-1 tumor antigen. Cancer Res. 2003;63:6948–55.
17. Rossig C, Nuchtern JG, Brenner MK. Selection of human antitumor single-chain Fv antibodies from the B-cell repertoire of patients immunized against autologous neuroblastoma. Med Pediatr Oncol. 2000;35:692–5.
18. Krengel U, Bousquet PA. Molecular recognition of gangliosides and their potential for cancer immunotherapies. Front Immunol. 2014;5:325. doi:10.3389/fimmu.2014.00325.
19. Schulz G, Cheresh DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. Cancer Res. 1984;44:5914–20.
20. Dobrenkov K, Ostrovnaya I, Gu J, Cheung IY, Cheung N-KV. Oncotargets GD2 and GD3 are highly expressed in sarcomas of children, adolescents, and young adults. Pediatr Blood Cancer. 2016;63(10):1780–5. doi:10.1002/pbc.26097.
21. Kailayangiri S, Altvater B, Meltzer J, Pscherer S, Luecke A, Dierkes C, et al. The ganglioside antigen GD2 is surface-expressed in Ewing sarcoma and allows for MHC-independent immune targeting. Br J Cancer. 2012;106:1123–33. doi:10.1038/bjc.2012.57.
22. Shurin GV, Shurin MR, Bykovskaia S, Shogan J, Lotze MT, Barksdale EM. Neuroblastoma-derived gangliosides inhibit dendritic cell generation and function. Cancer Res. 2001;61:363–9.
23. Yu AL, Gilman AL, Ozkaynak MF, London WB, Kreissman SG, Chen HX, et al. Anti-GD2 antibody with GM-CSF, interleukin-2, and isotretinoin for neuroblastoma. N Engl J Med. 2010;363:1324–34. doi:10.1056/NEJMoa0911123.
24. Simon T, Hero B, Faldum A, Handgretinger R, Schrappe M, Klingebiel T, et al. Long term outcome of high-risk neuroblastoma patients after immunotherapy with antibody ch14.18 or oral metronomic chemotherapy. BMC Cancer. 2011;11:21. doi:10.1186/1471-2407-11-21.
25. Gilman AL, Ozkaynak MF, Matthay KK, Krailo M, Yu AL, Gan J, et al. Phase I study of ch14.18 with granulocyte-macrophage colony-stimulating factor and interleukin-2 in children with neuroblastoma after autologous bone marrow transplantation or stem-cell rescue: a report from the Children's Oncology Group. J Clin Oncol. 2009;27:85–91. doi:10.1200/JCO.2006.10.3564.
26. Coughlin CM, Fleming MD, Carroll RG, Pawel BR, Hogarty MD, Shan X, Vance BA, Cohen JN, Jairaj S, Lord EM, Wexler MH, Danet-Desnoyers GH, Pinkus JL, Pinkus GS, Maris JM, Grupp SA, Vonderheide RH. Immunosurveillance and survivin-specific T-cell immunity in children with high-risk neuroblastoma. J Clin Oncol. 2006;24:5725–34. doi:10.1200/JCO.2005.05.3314.
27. Haworth KB, Arnold MA, Pierson CR, Leddon JL, Kurmashev DK, Swain HM, et al. Characterization of MHC class I and β-2-microglobulin expression in pediatric solid malignancies to guide selection of immune-based therapeutic trials. Pediatr Blood Cancer. 2016;63:618–26. doi:10.1002/pbc.25842.

28. Reid GSD, Shan X, Coughlin CM, Lassoued W, Pawel BR, Wexler LH, et al. Interferon-gamma-dependent infiltration of human T cells into neuroblastoma tumors in vivo. Clin Cancer Res. 2009;15:6602–8. doi:10.1158/1078-0432.CCR-09-0829.
29. Reichel J, Chadburn A, Rubinstein PG, Giulino-Roth L, Tam W, Liu Y, et al. Flow-sorting and exome sequencing reveals the oncogenome of primary Hodgkin and Reed-Sternberg cells. Blood. 2015;12:1061–72. doi:10.1182/blood-2014-11-610436.
30. Semeraro M, Rusakiewicz S, Minard-Colin V, Delahaye NF, Enot D, Vély F, et al. Clinical impact of the NKp30/B7-H6 axis in high-risk neuroblastoma patients. Sci Transl Med. 2015;7:283ra55. doi:10.1126/scitranslmed.aaa2327.
31. Stephanou A, Knight RA, Annicchiarico-Petruzzelli M, Finazzi-Agrò A, Lightmann SL, Melino G. Interleukin-1 beta and interleukin-6 mRNA are expressed in human glioblastoma and neuroblastoma cells respectively. Funct Neurol. 1992;7:129–33.
32. Silverman AM, Nakata R, Shimada H, Sposto R, DeClerck YA. A galectin-3-dependent pathway upregulates interleukin-6 in the microenvironment of human neuroblastoma. Cancer Res. 2012;72:2228–38. doi:10.1158/0008-5472.CAN-11-2165.
33. Ara T, Song L, Shimada H, Keshelava N, Russell HV, Metelitsa LS, et al. Interleukin-6 in the bone marrow microenvironment promotes the growth and survival of neuroblastoma cells. Cancer Res. 2009;69:329–37. doi:10.1158/0008-5472.CAN-08-0613.
34. Egler RA, Burlingame SM, Nuchtern JG, Russell HV. Interleukin-6 and soluble interleukin-6 receptor levels as markers of disease extent and prognosis in neuroblastoma. Clin Cancer Res. 2008;14:7028–34. doi:10.1158/1078-0432.CCR-07-5017.
35. Lagmay JP, London WB, Gross TG, Termuhlen A, Sullivan N, Axel A, et al. Prognostic significance of interleukin-6 single nucleotide polymorphism genotypes in neuroblastoma: rs1800795 (promoter) and rs8192284 (receptor). Clin Cancer Res. 2009;15:5234–9. doi:10.1158/1078-0432.CCR-08-2953.
36. Ferrer FA, Pantschenko AG, Miller LJ, Anderson K, Grunnet M, McKenna PH, et al. Angiogenesis and neuroblastomas: interleukin-8 and interleukin-8 receptor expression in human neuroblastoma. J Urol. 2000;164:1016–20.
37. Yang KD, Cheng SN, Wu NC, Shaio MF. Induction of interleukin-8 expression in neuroblastoma cells by retinoic acid: implication of leukocyte chemotaxis and activation. Pediatr Res. 1993;34:720–4. doi:10.1203/00006450-199312000-00005.
38. Lisignoli G, Toneguzzi S, Cattini L, Pozzi C, Facchini A. Different expression pattern of cytokine receptors by human osteosarcoma cell lines. Int J Oncol. 1998;12:899–903.
39. Morales-Arias J, Meyers PA, Bolontrade MF, Rodriguez N, Zhou Z, Reddy K, et al. Expression of granulocyte-colony-stimulating factor and its receptor in human Ewing sarcoma cells and patient tumor specimens: potential consequences of granulocyte-colony-stimulating factor administration. Cancer. 2007;110:1568–77. doi:10.1002/cncr.22964.
40. Ladenstein R, Pötschger U, Le Deley MC, Whelan J, Paulussen M, Oberlin O, et al. Primary disseminated multifocal Ewing sarcoma: results of the Euro-EWING 99 trial. J Clin Oncol. 2010;28:3284–91. doi:10.1200/JCO.2009.22.9864.
41. Berghuis D, Santos SJ, Baelde HJ, Taminiau AH, Egeler RM, Schilham MW, et al. Pro-inflammatory chemokine-chemokine receptor interactions within the Ewing sarcoma microenvironment determine CD8(+) T-lymphocyte infiltration and affect tumour progression. J Pathol. 2011;223:347–57. doi:10.1002/path.2819.
42. Song L, Ara T, Wu H-W, Woo C-W, Reynolds CP, Seeger RC, et al. Oncogene MYCN regulates localization of NKT cells to the site of disease in neuroblastoma. J Clin Invest. 2007;117:2702–12. doi:10.1172/JCI30751.
43. Dondero A, Pastorino F, Della Chiesa M, Corrias MV, Morandi F, Pistoia V, et al. PD-L1 expression in metastatic neuroblastoma as an additional mechanism for limiting immune surveillance. Oncoimmunology. 2015;5:e1064578. doi:10.1080/2162402X.2015.1064578.
44. Lussier DM, O'Neill L, Nieves LM, McAfee MS, Holechek SA, Collins AW, et al. Enhanced T-cell immunity to osteosarcoma through antibody blockade of PD-1/PD-L1 interactions. J Immunother. 2015;38:96–106. doi:10.1097/CJI.0000000000000065.

45. Routh JC, Grundy PE, Anderson JR, Retik AB, Kurek KC. B7-h1 as a biomarker for therapy failure in patients with favorable histology Wilms tumor. J Urol. 2013;189:1487–92. doi:10.1016/j.juro.2012.11.012.
46. Routh JC, Ashley RA, Sebo TJ, Lohse CM, Husmann DA, Kramer SA, et al. B7-H1 expression in Wilms tumor: correlation with tumor biology and disease recurrence. J Urol. 2008;179:1954–60. doi:10.1016/j.juro.2008.01.056.
47. Wang L, Zhang Q, Chen W, Shan B, Ding Y, Zhang G, et al. B7-H3 is overexpressed in patients suffering osteosarcoma and associated with tumor aggressiveness and metastasis. PLoS One. 2013;8:e70689. doi:10.1371/journal.pone.0070689.
48. Castriconi R, Dondero A, Augugliaro R, Cantoni C, Carnemolla B, Sementa AR, et al. Identification of 4Ig-B7-H3 as a neuroblastoma-associated molecule that exerts a protective role from an NK cell-mediated lysis. Proc Natl Acad Sci U S A. 2004;101:12640–5. doi:10.1073/pnas.0405025101.
49. Liebau C, Baltzer AWA, Schmidt S, Roesel C, Karreman C, Prisack JB, et al. Interleukin-12 and interleukin-18 induce indoleamine 2,3-dioxygenase (IDO) activity in human osteosarcoma cell lines independently from interferon-gamma. Anticancer Res. 2002;22:931–6.

Chapter 2
Overcoming Immune Suppression in the Tumor Microenvironment: Implications for Multi-modal Therapy

Theodore S. Johnson and David H. Munn

Abstract Effective immunotherapy, whether by checkpoint blockade, vaccines or adoptive cell therapy, is limited in most patients by a fundamental barrier: the immunosuppressive tumor microenvironment. This problem is more than just the suppression of effector T cells, but also includes profound defects in the inflammatory milieu and immunogenic antigen-presenting cells that are required to drive T cell activation. To date, much of the field of immunotherapy has focused on downstream checkpoints that regulate activated T cells, or on vaccination and T cell adoptive transfer to expand the T cell pool. Relatively less attention has been given to regulatory pathways that govern cross-presentation and response to endogenous tumor antigens. But these "upstream" pathways become particularly important in settings where immunotherapy is combined with standard-of-care chemotherapy or radiation therapy, both of which release a wave of tumor antigens. The choice of whether to treat these antigens as tolerizing or immunizing is fundamental to generating an effective immune response against the tumor. In this chapter we consider immunosuppressive mechanisms in the tumor microenvironment from the perspective of factors that that may impact the response to antigens from dying tumor cells.

Keywords Indoleamine 2,3-dioxygenase • IDO • Tolerance • Tumor microenvironment • Tumor • Immunotherapy • Checkpoint • Chemotherapy • Radiation

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2.1 Introduction

In this chapter, we will focus on two aspects of tumor immunotherapy that are particularly relevant to pediatrics, but which often receive somewhat less attention in the field. First, our emphasis will be less on the downstream checkpoints that affect activated T cells, and more on the fundamental upstream factors that control the cross-presentation of tumor antigens to T cells in the first place. It is increasingly being realized that this key endogenous antigen-presentation step needs to elicit robust T cell immunity in order for immunotherapy to be successful [1]. Unfortunately, however, the default response in the tumor is frequently T cell suppression and tolerance, rather than an aggressive response to tumor antigens. Thus, one of the important goals of tumor immunotherapy must be to re-configure the suppressive and tolerogenic tumor microenvironment so that it becomes robustly immunogenic for tumor antigen [2, 3].

The second, and conceptually related, focus of this chapter will be on ways in which immunotherapy can be integrated with standard-of-care chemotherapy and radiation-therapy treatment. In adult oncology, the combination of immunotherapy with chemo/radiation therapy is increasingly recognized as a potential opportunity (although currently under-utilized) for achieving valuable synergy [2, 4–6]. In pediatrics, however, it is virtually a requirement that immunotherapy be integrated in combination with the existing standard-of-care treatments. This is because in pediatrics the standard-of-care therapies are often highly effective, and even in relapsed or high-risk disease can still offer significant (albeit reduced) benefit. Thus, if immunotherapy is going to have a major near-term impact on the treatment of children, it will need to extend and enhance the efficacy of our existing treatments, not attempt to replace them. Fortunately, emerging preclinical evidence suggests that both chemotherapy and radiation are not only feasible for combination with immunotherapy, but can be highly synergistic.

2.2 Exploiting Immunotherapy to Create Synergy with Cytotoxic Therapy

2.2.1 Chemo-Immunotherapy: Beyond Synergy to True Synthetic Lethality

For a number of years it has been recognized that chemotherapy creates effects that can be exploited to enhance the immune response to tumors [7]. One obvious effect is the release of tumor antigens from dying cells; but, in addition, certain chemotherapy drugs may deplete regulatory T cells [8], or create lymphopenic conditions that favor T cell proliferation and expansion [9]. However, these effects are essentially passive: creating a general milieu in which vaccines or other immunotherapy may work better. A more active role for chemotherapy was revealed with the discovery of so-called “immunogenic cell death” (ICD) [10–12]. When certain preclinical

mouse tumor models are treated with particular chemotherapy drugs, the tumor cells die in a fashion that triggers a spontaneous immune response. Not only does this help prime the immune system against the tumor, but (at least in these particular model/drug combinations) a substantial component of the efficacy of the chemotherapy itself is actually contributed by the immune system [12]. While this was a ground-breaking discovery, in practical terms there are relatively few drugs that elicit ICD, and the effect is highly model-dependent [13]. Thus, while the underlying concept is important, the high-impact clinical role for immunogenic cell death is likely to be in combination with immunomodulatory agents that can enhance and exploit the effect [14]. As we will discuss, when the underlying inhibitory pathways are removed by active immunotherapy, then many chemotherapy drugs may prove to be immunogenic [4].

Ultimately, the goal in combining immunotherapy with chemotherapy is not merely "synergy" in the pharmacologic sense, but rather to generate authentic *synthetic lethality* by the combination. Synthetic lethality describes a combination in which the two agents together recruit an entirely new set of molecular mechanisms, which would not come into play with either agent alone [15, 16]. Thus, for example, in pre-clinical models, our own group has shown that combining a normally ineffective dose of chemotherapy with a specific immune-activating agent (i.e., an agent that blocks a tolerogenic checkpoint to dying tumor cells), allows the ineffective chemotherapy to now cause potent and rapid tumor regression [17]. The mechanism of anti-tumor effect was almost entirely immunologic (T cell dependent), but these immune mechanisms were only triggered if the tumor was also treated with chemotherapy.

2.2.2 The Importance of Endogenous Tumor Antigens

One of the surprising findings of the past several years has been the importance of endogenous tumor antigens in cancer immunotherapy [18]. Prior to the advent of checkpoint-blockade agents, the focus of immunotherapy was often on supplying antigens and T cells exogenously—e.g., via defined vaccines, TIL infusions, TCR-transgenic T cells, or CAR T cells. However, as increasing numbers of patients have been treated with blockade of the CTLA-4 and PD-1/PD-L1 pathways, it has become evident that the best responses are seen in those patients who have many mutational neoantigens in the tumor, and who already have a robust spontaneous immune response prior to treatment [19, 20].

In part this may simply be an artifact of early trials, which use only single-agent checkpoint blockade. In this setting, it is perhaps logical that only those patients who were already spontaneously pre-activated could respond to removing a single checkpoint. This effect may disappear as more powerful combination regimens are employed [21]. But the key take-home point is that the tumor's own endogenous antigens, cross-presented by the patient's own APCs to the endogenous T cell repertoire, were the critical factor that drove the anti-tumor response. This emphasizes the importance of endogenous tumor antigens, and the ability to cross-present them in an immunogenic fashion.

This has obvious importance for the immune response to chemotherapy or radiation, which release a wave of endogenous tumor antigens. But even in the case of an exogenous immune intervention, such as an antigen-specific vaccine or T cell adoptive transfer (CAR T cells, etc.), a successful long-term outcome may still depend on generating a response to endogenous tumor antigens [1]. Transferred T cells or defined vaccines are directed against just one or a few antigens. The initial response may be dramatic, but eventual emergence of escape variants is almost inevitable. If, however, during the initial period of robust inflammation and tumor killing, the endogenous host immune system becomes primed to endogenous tumor antigens, then the danger of escape variants is minimized, and long-term tumor control becomes a possibility.

2.2.3 Immunogenic Cell Death Versus Tolerogenic Cell Death: Overcoming Natural Pathways of Tolerance

The preceding general discussion does not tell us how—specifically—to render chemotherapy immunogenic in the clinic. In part this reflects the fact that much still needs to be discovered about the molecular mechanisms of combination chemo-immunotherapy. Also, our current options for immune intervention in the clinic are still somewhat limited, comprising primarily blocking agents against CTLA-4 or the PD-1/PD-L1 pathways, and blockade of the indoleamine 2,3-dioxygenase (IDO) pathway. However, the field is expanding rapidly and the armamentarium is quickly increasing. Thus, a better understanding of the molecular events that regulate the immune response following chemotherapy, in order to exploit this for therapy, has become a subject of some urgency.

In this regard, one fundamental insight emerging recently is the fact that the immune response to dying cells—even normal, non-malignant self cells—is not fixed and inherent. Rather, it reflects a combination of signals generated by the manner in which the cells die (ICD, apoptosis, necrosis etc.), combined with signals from the milieu in which the dying cells are cross-presented by the immune system. These local environmental signals are a very active—and changeable—process. Blocking even one of the tolerogenic signals elicited by apoptotic cells may render dying cells suddenly immunogenic instead. Thus, for example, the tolerogenic IDO pathway is strongly up-regulated by exposure to apoptotic cells [22]. When challenged with apoptotic self cells, normal IDO-sufficient mice remained tolerant, but mice lacking the IDO1 gene rapidly developed lethal lupus-like autoimmunity against self antigens [22–24]. Thus, it was not the nature of the antigens themselves that determined immunity versus tolerance, nor the type of cell death; but rather the ability of the apoptotic cells to elicit the immunosuppressive IDO signal. If this IDO pathway was blocked, then the same cells, and the same self antigens, now became immunogenic.

The relevance of this concept for cancer treatment is that chemotherapy and radiation release a wave of tumor antigens, many of which are potentially immunogenic

[18, 25]. The problem is that these antigens are released into a tumor milieu that is overwhelmingly dominated by immunosuppressive mechanisms. Thus, even though dying tumor cells are potentially immunogenic [6] the actual outcome is usually tolerance and anergy, due to these dominant suppressive mechanisms. If, however, the tolerogenic pathways used by the tumor (such as IDO, Tregs or others) can be identified and blocked at the time of chemotherapy, then the antigens thus released may be treated as immunizing instead of tolerizing. This concept is now well accepted in principle [1, 2], and relevant preclinical studies are beginning to emerge [17], but much of the underlying molecular machinery still remains to be discovered. However, even with our current limited state of knowledge, it is possible to begin to design clinical trials aimed at exploiting the immunogenicity of chemotherapy.

2.3 Negative Regulation in the Tumor Microenvironment

In this section we will briefly discuss several of the key suppressive pathways operating in the tumor. Many of these are discussed in detail elsewhere in this volume, so our focus here is specifically how these inhibitory pathways may affect the cross-presentation and immune response to tumor antigens.

2.3.1 Regulatory T Cells: Recruitment and Activation

Regulatory T cells (Tregs) in tumors are an important suppressive population [26]. Physically depleting Tregs [27] or inhibiting the signals that they require [28] rescues anti-tumor immune surveillance. However, it is still unclear how Tregs exert their suppressive function. One important mechanism may be their ability to inhibit tumor-associated antigen-presenting cells [29, 30]. This would be a key leverage point for control of antigen cross-presentation to T cells.

One important unanswered question in the field is why Treg activity is so excessive in the tumor. Many of the Tregs in tumors appear to recognize the same self antigens as in normal tissues [31], but there is a greater degree of constitutive functional activation of Tregs in tumors [32]. Several upstream pathways are known to activate tumor-associated Tregs, includes IDO [32] and neuropilin-1 [33]. Recently, it was shown that when Tregs are activated by IDO they up regulate the PD-1 receptor; PD-1 signaling then maintains the suppressive Treg phenotype long-term, via activation of the downstream PTEN phosphatase [17]. Neuropilin-1 also activates PTEN in Tregs [33], and PTEN has been recently implicated in maintaining normal function and stability of Tregs in the normal immune system [34, 35]. Thus, PTEN may be a centrally-positioned pathway in tumor-induced activation of Tregs. In tumor-bearing mice, ablation or inhibition of the PTEN pathway in Tregs prevented tumors from creating their usual immunosuppressive microenvironment, and this markedly enhanced the immune response to dying tumor cells following chemotherapy [17].