Explaining the Paucity of Intratumoral T Cells: A Construction Out of Known Entities

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This essay addresses the question of how tumors escape control by the immune system. The literature strongly points to inadequate accumulation of T cells among cancer cells as being the proximate cause, but this observation has no acceptable explanation as yet. An approach to this problem is adopted wherein the chemokines and chemokine receptors that normally mediate the trafficking of T cells to inflamed tissues are reviewed and considered in the context of their relative levels of expression in a transplanted colorectal tumor model. This method of reasoning—consistent with Bertrand Russell's (1985) advice, "Whenever possible, substitute constructions out of known entities for inferences to unknown entities"—leads to the proposal that signaling via the chemokine receptor, CXCR4, impairs the function of CXCR3 on the immune cells that are responsible for suppressing the growth of cancers.

The immune system has the capacity to control the growth of cancer. Clinical trials in which patients with certain types of solid tumors were administered neutralizing antibodies to membrane proteins that are expressed on activated effector and regulatory T cells, such as CTLA-4 and PD-1, or to PD-L1, the ligand for PD-1 that is expressed by some myelomonocytic cells and cancer cells, have shown that some patients show striking and often durable remissions (Hodi et al. 2010; Brahmer et al. 2012; Topalian et al. 2012; Hamid et al. 2013; Wolchok et al. 2013; Herbst et al. 2014; Powles et al. 2014; Tumeh et al. 2014; Motzer et al. 2015; Ferris et al. 2016). Other studies in which in vitro expanded tumor-infiltrating T cells have been adoptively transferred to cancer patients also have documented regressions of tumors (Tran et al. 2016). Finally, adoptive transfer of T cells expressing chimeric antigen receptors, so-called CAR T cells, has been an effective therapy for CD19-expressing B-cell malignancies (Porter et al. 2011). Thus, as predicted by earlier studies, such as that showing better long-term outcomes in colorectal cancer patients whose tumors were characterized by "CD8⁺ T cells infiltrated within cancer cell nests" (Naito et al. 1998), the immune system, through the cytotoxic activities of the CD8⁺ T cell, has the capacity to kill immunogenic cancer cells. These successes present tumor immunologists with an urgent responsibility now to understand why only subsets of patients with only some types of cancers respond to these forms of immunotherapy. That is to say, "Why is immunotherapy ineffective in most patients?"

Potential contemporary answers to this question range from being self-evident, although nonetheless important, such as whether cancer cells have induced an adaptive

immune response, to others for which mechanistic explanations are still evolving, such as T-cell "exhaustion" and immune inhibition by "myeloid-derived suppressor cells". Although the former can be definitively resolved by experimental approaches like the development of assays of anticancer T-cell immunity, the latter explanations will be more difficult to affirm or disprove. The complexity of the vertebrate immune system presents the tumor immunologist with almost too many experimental opportunities to "validate" hypotheses that may have conceptual rather than molecular definitions. More relevant to this question of tumoral immune escape is our knowledge that although the immune system evolved to defend against an extraordinary array of pathogens, it also developed "brakes" that prevent attack of semiallogeneic fetuses and autoimmune diseases. Namely, it is "normal" that the adaptive immune system may not always damage immunogenic tissue. For this reason, we should not assume that the capacity of an immunogenic tumor to escape immune control is a consequence of an abnormal immunological process, like a hostile tumor microenvironment (TME) causing "T-cell exhaustion." Instead, this unfortunate clinical circumstance may be explained by an immune pathway that benefits the host in biological circumstances other than cancer.

T-CELL EXCLUSION—THE SHARED CHARACTERISTIC OF TUMORS ESCAPING IMMUNE CONTROL

The most striking difference between a tissue immune reaction eliminates epithelial cells expressing immuno-

genic antigens, as in influenza virus–infected pulmonary epithelium, and an ineffective tumor immune reaction is the relative numbers of infiltrating T cells. Although informative and discerning metrics have been devised, such as “immunoscore” (Galon et al. 2016), and scientifically vague, but descriptive terms, such as “hot” and “cold” tumors, have been promoted to exemplify this difference, the most unambiguous characteristic of an immunologically uncontrolled tumor is the relative paucity of T cells that are juxtaposed to cancer cells (Joyce and Fearon 2015). In contrast, tumors that are being effectively eliminated by an immune response have dense infiltrates of CD8⁺ T cells, as do the islets of Langerhans of the pancreas in autoimmune type I diabetes mellitus, organs undergoing cell-mediated immune rejection, and, indeed, virally infected tissues, the last being a biological circumstance that selected this CD8⁺ T-cell response. Therefore, if the relative intensity of the intratumoral accumulation of CD8⁺ T cells is the critical variable that distinguishes between effective and ineffective antitumor immune reactions, one should determine whether the principles that have been discovered to regulate these other examples of CD8⁺ T-cell accumulation also pertain to cancer. This approach would be consistent with the admonition of Bertrand Russell: “Whenever possible, substitute constructions out of known entities for inferences to unknown entities” (Russell 1985).

THE NETWORK OF CHEMOKINE AND CHEMOKINE RECEPTORS: CXCR3 AS THE MEDIATOR OF INTRATUMORAL T-CELL ACCUMULATION

We have known for many years that an extensive network of chemokines and chemokine receptors regulates the trafficking of immune cells (Griffith et al. 2014). The first chemokine receptor, which is thought to be CXCR4, appears in the jawless fish, lamprey, an ancient example of the vertebrate lineage in which the adaptive immune system that is typified by the *RAG* genes is absent (Kuroda et al. 2003). With the further evolution of vertebrates, there is an explosion of chemokines and chemokine receptors, such that the zebrafish has 63 genes encoding chemokines and 24 genes encoding chemokine receptors (DeVries et al. 2006). The relevance of this system for directing the cellular migration is not limited to the immune system and is essential also for the development of many organs. However, in the postnatal period, the primary role of this system may well be immunological. The cells of the immune system most often mediate their effector functions in tissues that are remote from their sites of development and residence, the bone marrow, thymus, and secondary lymphoid organs. With respect to the effector CD8⁺ T cell, a remarkably consistent model has been developed. After their initial clonal expansion in secondary lymphoid organs, effector CD8⁺ T cells are released into the peripheral blood. Although these effector cells up-regulate their expression of a number of chemokine receptors during differentiation from naïve to

effector CD8⁺ T cell, a single receptor, CXCR3, appears to be most often responsible for mediating homing to sites of inflammation. These CXCR3-mediated CD8⁺ T-cell responses depend on the production in the inflamed tissues of the chemokines, CXCL9, CXCL10, and CXCL11, the levels of which are regulated by type I and type II interferon (IFN) (Griffith et al. 2014). A dominant role for CXCR3 in T-cell homing has been found for a range of biological circumstances in which target cells express major histocompatibility complex (MHC) class I/peptide complexes bearing neoepitopes, including allograft rejection (Seung et al. 2011), viral infections (Klein et al. 2005; Hsieh et al. 2006), autoimmunity (Frigerio et al. 2002), and cancer (Mikucki et al. 2015), the last example being especially relevant to the subject of this discourse. In the B16 mouse model of melanoma, CXCR3 was shown to have a nonredundant role in mediating the intratumoral accumulation of adoptively transferred, cancer-specific, effector CD8⁺ T cells, which resulted in control of tumor growth by two complementary approaches—use of CD8⁺ T cells in which the *CXCR3* gene had been disabled and administration of neutralizing anti-CXCR3 antibody. Two other receptors, CCR2 and CCR5, did not support the intratumoral trafficking of CD8⁺ T cells in this experimental model, serving, in effect, as negative controls. Therefore, despite the multiplicity of chemokine receptors that an effector CD8⁺ T cell expresses, and regardless of a diversity of immunological tissue reactions, CXCR3 has a dominant, positive role in the trafficking of these cells. In this regard, it is interesting to note that other immune cell types that should be considered important for orchestrating an effective antitumor immune reaction—TH1 cells, the subset of dendritic cells (DCs) with antigen cross-presenting capability, and natural killer (NK) cells—also express CXCR3, whereas other cell types that may even have tissue protective functions, such as monocytes and macrophages, do not.

Because the response of the preclinical B16 melanoma mouse model to anti-CTLA-4 immunotherapy (Leach et al. 1996) has been predictive of clinical immunotherapeutic responses of human melanoma (Hodi et al. 2010), the absence of a clinical response and the paucity of intratumoral T cells in those cancers that do not respond to immunotherapy may be considered to be caused by an absence of signaling by CXCR3 on CD8⁺ T cells within the TME. This deficiency may have one obvious explanation, which would be insufficient level of intratumoral CXCL9, CXCL10, or CXCL11, the ligands for CXCR3, but a second explanation is also possible, which is that the TME impairs signaling by CXCR3. There is evidence in support of the first explanation with the finding of epigenetic silencing of CXCL9 and CXCL10 as a means by which a preclinical tumor model circumvents immune control (Peng et al. 2015). Epigenetic silencing of the expression of these chemokines has been proposed also as the mechanism by which the semiallogeneic fetus escapes rejection by the maternal adaptive immune response (Nancy et al. 2012). Moreover, the finding that the type I IFN response is necessary for immune control

of immunogenic mouse tumors (Diamond et al. 2011; Fuertes et al. 2011) may be related to the capacity of this cytokine to induce the transcription of CXCL10. Thus, it is reasonable to propose that insufficient levels of the CXCR3-related chemokines account for the impaired intratumoral accumulation of T cells. However, there may also be evidence in support of impaired CXCR3 function on immune cells in the TME.

IMPAIRED FUNCTION OF CXCR3 IN THE C26 COLORECTAL TUMOR MODEL

The C26 tumor is a chemically induced, transplantable mouse colon carcinoma that has been used in studies of cancer-induced cachexia. Its origin as a mutagen-induced cancer may indicate that it expresses neoantigens for presentation via the MHC class I pathway when transplanted and grown in mice. Therefore, it may serve as an appropriate model to explore the mechanism of impaired intratumoral accumulation of cancer-specific CD8⁺ T cells. In our studies of cancer-induced cachexia, we found relatively high mRNA levels for CXCL9 and CXCL10 in analyses of transplanted C26 tumors (Flint et al. 2016), which indicated that these tumors had two elements of an antitumor immune response, immunogenicity and CXCR3-related chemokines, that might be anticipated to be sufficient for immune control tumor growth. However, control obviously does not happen and ectopic C26 tumors continue to grow in immunocompetent, syngeneic mice. To gain insight into the reasons for this escape from immune control, we performed RNA-seq on whole C26 tumors to assess the expression of genes that mark specific immune cell types (Table 1). The C26 tumor has abundant monocytes and/or macrophages, as exemplified by the high mRNA levels of *CSF1R*, *ITGAM*, and *F4/80*. In contrast, and assuming that the expression of each gene per cell is relatively equivalent, the frequency of T cells is only 1%–5% that of monocytes/macrophages, based on the low mRNA levels of *CD3e*, *CD4*, and *CD8a*. Also, the mRNA levels of *BATF3*, *CD103*, *XCR1*, *CLEC9a*, and *FLT3*, five genes expressed by the antigen crossing-presenting subset of DCs, are similarly low, as is the mRNA level for the gene, *NCR1* (NKp46), that encodes a receptor that is uniquely expressed by NK cells. Therefore, in the C26 tumor, there is a relative paucity of precisely those immune cells that would be capable of

Table 1. Expression of genes denoting immune cell types in the C26 tumor

Gene	Cell type	RPKM
<i>CSF1R</i>	Mono, Mac	2652
<i>ITGAM</i>	Mono, Mac	2901
<i>F4/80</i>	Mac	1427
<i>CD3e</i>	T cell	33
<i>CD4</i>	T cell	69
<i>FOXP3</i>	Treg cell	14
<i>CD8a</i>	T cell, BATF3 ⁺ DC	117
<i>BATF3</i>	BATF3 ⁺ DC	29
<i>CD103</i>	BATF3 ⁺ DC	22
<i>XCR1</i>	BATF3 ⁺ DC	31
<i>CLEC9a</i>	BATF3 ⁺ DC	22
<i>FLT3</i>	BATF3 ⁺ DC	15
<i>NCR1</i>	NK cell	35

RPKM, reads per kilobase of transcript per million mapped reads; Mono, monocyte; Mac, macrophage; Treg, T regulatory; DC, dendritic cell; NK, natural killer.

promoting the killing of cancer cells: CTLs, NK cells, and the subset of DCs that direct clonal expansion of cancer-specific CD8⁺ T cells within the draining lymph node and within the tumor itself.

An examination of the expression of chemokines and chemokine receptors in the C26 tumor reveals a pattern that could account for the marked difference in immune cell types that accumulate in the C26 tumor. The mRNA for CXCR4, which is expressed by all immune cell types, is relatively abundant, presumably reflecting the presence of monocytes and macrophages (Table 2). In accord with this surmise, the mRNA levels for CCR2 and CCR5, two chemokine receptors that are expressed by monocytes and macrophages, are high. In contrast, mRNA levels for CXCR3 are drastically lower. This finding is consistent with the presumed low frequency of T cells, BATF3⁺ DCs, and NK cells in the C26 tumor because these three cell types, in contrast to monocytes and macrophages, express CXCR3. The paucity of CXCR3-expressing immune cells cannot be explained by low tumoral expression of the ligands for this receptor because the combined mRNA level of CXCL9, CXCL10, and CXCL11 is even higher than that of CCL2 mRNA, the ligand for CCR2. This circumstance, assuming that these transcripts are translated, excludes the possibility that the relative deficiency of CXCR3-expressing cells is secondary to low levels if the receptor's cognate chemokines. Indeed, if

Table 2. Expression of chemokine and chemokine receptor genes in the C26 tumor

Receptor	Cell types	RPKM	Chemokine	RPKM	Chemokine-R/chemokine ratio
CCR2	Teff, Mono, Mac	825	CCL2	1125	0.7
CCR5	Teff, iNKT, NK, Mac, pDC	1775	CCL3	42	7.7
			CCL4	55	
			CCL5	133	
CXCR3	TH1, CTL, pDC, Batf3 ⁺ DC, iNKT, NK, ILC1	10	CXCL9	1384	0.005
			CXCL10	473	
			CXCL11	68	
CXCR4	All	308	CXCL12	187	1.7

RPKM, reads per kilobase of transcript per million mapped reads; Teff, effector T-cell; Mono, monocyte; Mac, macrophage; iNKT, invariant natural killer T; pDC, plasmacytoid dendritic cell; CTL, cytotoxic T lymphocyte.

one calculates the ratio of the mRNA levels of each chemokine receptor to its associated chemokine(s) as a measure of the activity of the receptor, one finds that the function of CXCR3 may be <1% that of CCR2 in the C26 tumor. This finding is compelling evidence in support of the possibility that the CXCR3-expressing cells may not accumulate in the C26 tumor even when CXCL9-11 are expressed and strongly suggests that some aspect of the TME impairs CXCR3 function and causes an intratumoral immune phenotype that is equivalent to that of B16 melanoma-bearing mice treated in which cancer-specific CD8⁺ T cells are lacking CXCR3.

CXCR4 AS THE CHEMOKINE RECEPTOR THAT INHIBITS CXCR3

By conforming to the philosophical axiom of Bertrand Russell and focusing on the “known entity” of the chemokine/chemokine receptor system, we have identified a disparity between the intratumoral mRNA levels of CXCR3 and its ligands that strongly suggests that the TME suppresses the function of this chemokine receptor. Again, we turn to the literature in seeking a potential explanation of how the TME might alter the function of CXCR3. A spontaneously immunogenic, autochthonous mouse model of pancreatic ductal adenocarcinoma (PDAC) (Hingorani et al. 2005) escapes immune control by excluding T cells from the vicinity of cancer cells (Feig et al. 2013). Several findings suggested the possibility that CXCR4, a chemokine receptor found on all immune cells, had a role in this T-cell exclusion phenomenon: Conditional depletion of the cancer-associated fibroblasts enabled T-cell-dependent suppression of tumor growth; the cancer-associated fibroblasts were the major, intratumoral cellular source of CXCL12, the ligand for CXCR4; and the PDAC cancer cells appeared to be “coated” with the chemokine, despite the absence of CXCL12 mRNA in these cells (Kraman et al. 2010; Feig et al. 2013). A potential immunosuppressive role for CXCR4 was assessed by treating PDAC-bearing mice with AMD3100, a small-molecule inhibitor of CXCR4. Within 24 h of treatment initiation, T cells had accumulated among cancer cells, and within 48 h, in combination with a T-cell checkpoint antagonist, anti-PD-L1 antibody, to which mouse and human PDACs are consistently resistant, the mouse tumor volume had decreased by 15%. If the many publications attesting to the inhibitory specificity of AMD3100 for CXCR4 are correct, and if this PDAC tumor model obeys the same, nonredundant requirement for CXCR3 expression and function by CD8⁺ T cells in the B16 mouse melanoma (Mikucki et al. 2015), one is led to the conclusion that CXCR4 signaling suppresses the intratumoral function of CXCR3 in mouse PDAC to mediate the exclusion of T cells. The finding that AMD3100 also led to immune control of an ectopic Lewis lung tumor expressing the antigen, ovalbumin (Feig et al. 2013), indicates that this conclusion is not restricted to mouse PDAC. (See Fig. 1.)

CXCR4 has several characteristics that distinguish it from the other leukocyte, Gαi protein-coupled receptors (GPCRs) that mediate chemotaxis. An ortholog of CXCR4 exists in the lamprey (Kuroda et al. 2003), a member of the Agnatha class. Therefore, this chemokine receptor, uniquely among all chemokine receptors, evolved before the vertebrate adaptive immune system of immunoglobulin and T-cell receptors. CXCR4 and its CXCL12 ligand demonstrate the highest degree of evolutionary conservation when chemokines and chemokine receptor sequences of human, mouse, chicken, frog, zebrafish, and pufferfish are compared (DeVries et al. 2006). Furthermore, unlike other chemokine receptors, CXCR4 has important nonimmunological roles, such as in the development of neural (Zou et al. 1996; Chalasani et al. 2003; Haas and Gilmour 2006) and cardiovascular systems (Tachibana et al. 1998), germ cell migration (Knaut et al. 2003; Molyneaux et al. 2003), and hematopoiesis (Zou et al. 1996). These features have led some investigators to classify CXCR4 and its ligand, CXCL12, as having “homeostatic” functions, in contrast to the immune and inflammatory functions of the other leukocyte chemokine receptors. Seen from this perspective, it is reasonable to consider the role of CXCR4 in other organ systems to obtain hints concerning whether and how it may alter the function of CXCR3 on immune cells.

An early demonstration of CXCR4 signaling altering the function of another GPCR was the finding that the chemotactic activities of both the μ- and δ-opioid GPCRs in several cell types were desensitized following activation of CXCR4 in the responding cells (Szabo et al. 2002). Interestingly, this study also found that pretreatment with CXCL12 followed by opioid administration into the periaqueductal gray matter of the brain increased a behavioral response to a painful stimulus, consistent with CXCR4 signaling suppressing opioid receptor signaling of a cellular response distinct from motility. This conclusion was confirmed and extended by the demonstration that CXCR4, CXCL12, and the μ-opioid receptor were coexpressed by individual neurons in several regions of the brain areas (Heinisch et al. 2011). Moreover,

Figure 1. Proposed basis for the exclusion of T cells from tumors. CXCL12 “coats” tumor cells and stimulates CXCR4 on T cells, dendritic cells (DCs), and natural killer (NK) cells. CXCR4 then inhibits the capacity of CXCR3 to respond to tumor-derived CXCL9, CXCL10, and CXCL11.

whole patch-clamp recordings of the periaqueductal grey neurons showed that CXCL12 blocked morphine-induced electrophysiological effects. This effect of CXCL12 was suppressed by AMD3100, demonstrating that it was mediated by CXCR4.

CXCR4 may mediate these inhibitory effects on opioid receptor function as a consequence of these two receptors forming heteromeric complexes (Stephens and Handel 2013). Therefore, in the context of this discourse on tumor immunity, it is of some interest that CXCR4 forms hetero-oligomeric complexes with CCR2 and CCR5 (Sohy et al. 2007, 2009). In the heterodimer formed between CXCR4 and CCR2, there is negative binding cooperativity between the subunits such that ligands that are specific for one receptor suppress the binding of the ligand to the other receptor. A similar negative binding cooperativity has been demonstrated for the chemokines interacting with heterodimers formed between CXCR4 and CCR5. The basis for such negative binding cooperativity was not determined but was suggested to be a consequence of allosteric modulation across the receptor heteromer interface. Although there is no evidence that CCR2 or CCR5 mediate the intratumoral accumulation of T cells, and, in fact, there is a report that CCR5 does not mediate this process (Mikucki et al. 2015), these findings do show the capacity of CXCL12 to cause CXCR4 to suppress the signaling by another chemokine receptor. In this respect, then, it may be relevant that CXCR4 and CXCR3 have been shown to form heteromeric complexes when ectopically expressed in HEK293T cells (Watts et al. 2013). Negative binding cooperativity between CXCR4 and CXCR3, however, was not observed in intact HEK293T cells, although the analysis was not performed with cells that normally express these two receptors.

These studies of the interaction of ligands with CXCR4–CCR2–CCR5 chemokine receptor heteromers also revealed that the effects of inhibitors of CXCR4 are more complex than has been concluded based on standard experiments using cells expressing only single types of chemokine receptors. For example, treatment of cells expressing only CCR2 with the CXCR4 antagonist, AMD3100, does not affect the binding of CCL2, and treating cells expressing only CXCR4 inhibits binding of CXCL12. With cells expressing CXCR4 in addition to CCR2, however, AMD3100 treatment suppresses not only binding of CXCL12 to CXCR4, but also that of CCL2 to CCR2 (Sohy et al. 2009). Similarly, AMD3100 does not alter the binding of CCL4 to cells expressing only CCR5, but the CXCR4 antagonist does alter the kinetics of the interaction between CCR5 and CCL4 with cells that also express CXCR4. These effects, which may be secondary to the partial agonistic activity of AMD3100 on CXCR4 (Zhang et al. 2002), emphasize the necessity to conduct *in vitro* experiments that reflect at least some aspects of the realities of *in vivo* biology—namely, that cells rarely express only a single chemokine receptor. For example, populations of activated effector CD8⁺ T cells may express CCR2, CCR5, CXCR3, CXCR4, and CXCR6. Studies of chemokine receptor antagonists must determine which inhibitors are truly “spe-

cific” for their target receptor and do not alter the function of potential heteromeric partners.

Finally, CXCL12, the ligand of CXCR4, also shows a complexity that distinguishes it from other chemokines, especially with respect to the form in which it is present in carcinomas. In the autochthonous mouse model of PDAC, cancer cells stain with anti-CXCL12 (Feig et al. 2013). This staining with anti-CXCL12 of cancer cells has been reported also for human ovarian cancer (Scotton et al. 2002; Jiang et al. 2006), colorectal cancer (Akishima-Fukasawa et al. 2009), and PDAC (Liang et al. 2010), and we have observed it in mouse and human non–small cell lung cancer (NSCLC). These observations may be relevant to the means by which these cancers escape immune control because they are all characterized by exclusion of T cells from the vicinity of cancer cells (Naito et al. 1998; Zhang et al. 2003; Salmon et al. 2012; Feig et al. 2013) except for relatively infrequent examples of NSCLC and colorectal cancer that are associated with a high somatic mutational burden (Le et al. 2015; Rizvi et al. 2015). The means by which CXCL12 associates with cancer cells is not known, but it is reasonable to consider the possibility that this cell surface CXCL12 will be presented to CXCR4-expressing cells in a multimeric form rather than the monomeric form that is typically used in *in vitro* studies. Recent studies suggest that monomeric CXCL12 may not be the only or even most prevalent form of the chemokine. For example, dimeric CXCL12 is induced by the interaction of monomeric CXCL12 with negatively charged glycosaminoglycans (Veldkamp et al. 2005), which are a ubiquitous constituent of the TME. Dimeric CXCL12, in contrast to monomeric CXCL12, has been found to be resistant to proteolytic inactivation (Takekoshi et al. 2012), suggesting a means by which the half-life of the chemokine may be extended in a tissue-specific manner. Furthermore, dimeric CXCL12 elicits CXCR4-dependent Ca²⁺ flux, adenylyl cyclase inhibition, and activation of ERK1/2, but not chemotaxis (Drury et al. 2011). The interaction of dimeric CXCL12 with CXCR4 is also influenced by posttranslational sulfation of extracellular tyrosines (Veldkamp et al. 2006, 2008; Seibert et al. 2008). Thus, the physical state of CXCL12 may be dictated by the TME, and this may be an additional way that a tumor determines the type of intracellular signals that are elicited by CXCR4, adding an additional element of biological flexibility to this unusual chemokine/chemokine receptor system.

CONCLUSION

Immunogenic carcinomas escape immune control by creating a microenvironment that prevents the accumulation of T cells, DCs, and NK cells. Signaling by CXCR3 may usually mediate the accumulation of these immune cell types in tissue responses associated with the killing of cells expressing antigens, suggesting that the relative absence of these immune cell types in tumors is secondary either to a deficiency of the CXCR3-associated chemo-

kines or to the malfunction of the chemokine receptor. An example is presented of a TME in which there is abundant expression of the CXCR3-associated chemokines, CXCL9, CXCL10, and CXCL11, but a relative paucity of T cells, DCs, and NK cells. The absence of CXCR3-expressing immune cells in this tumor, then, must be secondary to inhibition of CXCR3 function. Because suppressing signaling by CXCR4 leads to intratumoral accumulation of T cells in this and other tumor models, because CXCL12 is present in tumors, and because the depletion of the intratumoral stromal cells that produce CXCL12 leads to control of tumor growth, one may reason that CXCR4 is responsible for the inhibition of CXCR3 function. This inhibition may be mediated through a physical association of CXCR4 with CXCR3 because ligation of CXCR4 in heteromeric complexes containing another GPCR may inhibit the function of the partner chemokine receptor. This type of cross-desensitization may occur also with CXCR4 “antagonists” that have partial agonistic activity, which may compromise the therapeutic efficacy of these antagonists in overcoming CXCR4-mediated restriction on the intratumoral accumulation of T cells.

This essay opened with a quote from Bertrand Russell and, in closing, one is reminded of another comment from this philosopher/mathematician: “The method of ‘postulating’ what we want has many advantages; they are the same as the advantages of theft over honest toil. Let us leave them to others and proceed with our honest toil” (Russell 1919). “Honest toil” in this essay refers to the experiments that have been performed by many thoughtful scientists whose experiments have provided the careful measurements of biological reactions that have enabled “constructions of known entities.” Whether the author has deviated too far in the direction of “postulating” what he “wants” is a question that, thankfully, can be answered by experimentation.

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