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EDITORIAL

Microbiota-associated immunotherapy resistance caused by deficient PD-L2 - RGMb signaling

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ABSTRACT

In a recent paper in *Nature*, Park *et al*. propose a mechanism through which intestinal dysbiosis compromises the efficacy of immunotherapy targeting the PD-L1/PD−1 interaction. Dysbiosis may upregulate a pair of checkpoint molecules, i.e. PD-L2 interacting with RGMb. Antibodies targeting PD-L2/RGMb can restore responses to PD−1 blockade in the context of dysbiosis.

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Shifts in the microbiota toward a pathogenic disequilibrium (dysbiosis) accompany aging, as well as most major diseases including cancer[1]. Of note, cancers evolving on immunocompetent mice that are germ-free or that have been treated with a cocktail of broad-spectrum antibiotics (ABX) fail to respond to PD−1 or PD-L1 blockade unless they receive fecal microbial transplantation (FMT) from normal specific-pathogen-free (SPF) mice or from human donors bearing a normal (eubiotic) microbiota[2]. This appears clinically relevant because, in melanoma patients that are refractory to PD−1/PD-L1 blockade, FMT can restore the response to immunotherapy[3,4].

Having replicated the aforementioned result in C57BL/6 mice bearing ectopic (subcutaneous) MC38 colon carcinomas, Park *et al*. showed that, even before immunotherapy targeting PD-L1, the mesenteric (mLN) and tumor-draining lymph nodes (tdLN) from dysbiotic (germ-free or ABX-treated) but not from eubiotic mice exhibit the upregulation of PD-L2 on $CD11b^+MHCII^+$ and $CD11c^+MHCII^+$ cells. This appears to be mechanistically important to explain the failing efficacy of PD-L1 blockade against MC38 tumors in dysbiotic mice because a combination of PD-L1 plus PD-L2 blockade restores therapeutic responses in dysbiosis, but is not superior to PD-L1 blockade alone in eubiosis. Subsequent comparative analysis of FMTs from complete responder or non-responder patients into mice, metagenomic sequencing of the reconstituted microbiota, antibiotic selections and culturomics led to the identification of two species (*Coprobacillus cateniformis* and *Erysipelatoclostridium ramosum*) that enhanced PD-L1 blockade-induced immunotherapy responses when monocolonized into mice[5].

Monocolonization of germ-free mice with *C. cateniformis* (but not *E. ramosum*) was able to downregulate PD-L2 on myeloid cells. Likewise, soluble surface extracts from *C. cateniformis*, downregulated PD-L2 expression on bone marrow-derived dendritic cells (DCs), highlighting the multifaceted impact of gut bacteria on the immune function during immunotherapy. When such soluble surface extracts were pulsed onto DCs, DCs increased their capacity to stimulate $CD8^+$ T cells in vitro, and this immunostimulatory effect was lost if the DCs were transduced with a vector increasing PD-L2 expression. Similarly, compared to wildtype DCs, *C. cateniformis* soluble surface extracts pre-treated DCs or DCs manipulated to lack PD-L2 expression injected into melanomas expressing the model antigen ovalbumin (OVA) were more efficient in controlling tumors established in SPF mice. Moreover, while in sterile mice, administration of PD-L2 overexpressing DCs exacerbated tumor growth, *C. cateniformis* monocolonized mice allowed an anti-tumor effect that could be reversed by this enforced PD-L2 expression on DCs. This kind of epistatic experimentation suggests that microbiota-modulated variations in PD-L2 expression on DCs are indeed determinants for the efficacy of immunotherapy. Additionally, the synergetic anti-tumor activity of PD-L1 plus PD-L2 blockade pointed out that PD-L2 effect was mediated by interaction with another PD-L2 receptor: RGMb (*Repulsive Guidance Molecule b*)

In a further twist, Park et *al*. compared the immunotherapeutic efficacy of two different PD-L2 antibodies, one that blocks the interaction of PD-L2 with both PD−1 and RGMb and another that only blocks the interaction with RGMb. Both antibodies, as well as an antibody specific to RGMb, were all found to similarly improve the efficacy of either PD−1 or PD-L1 blockade against MC38 cancer in dysbiotic mice. Thus, it appears that the PD-L2-RGMb interaction (but not the interaction between PD-L2 and PD−1) must be targeted to improve the efficacy of PD-L1 blockade in the context of dysbiosis. Of

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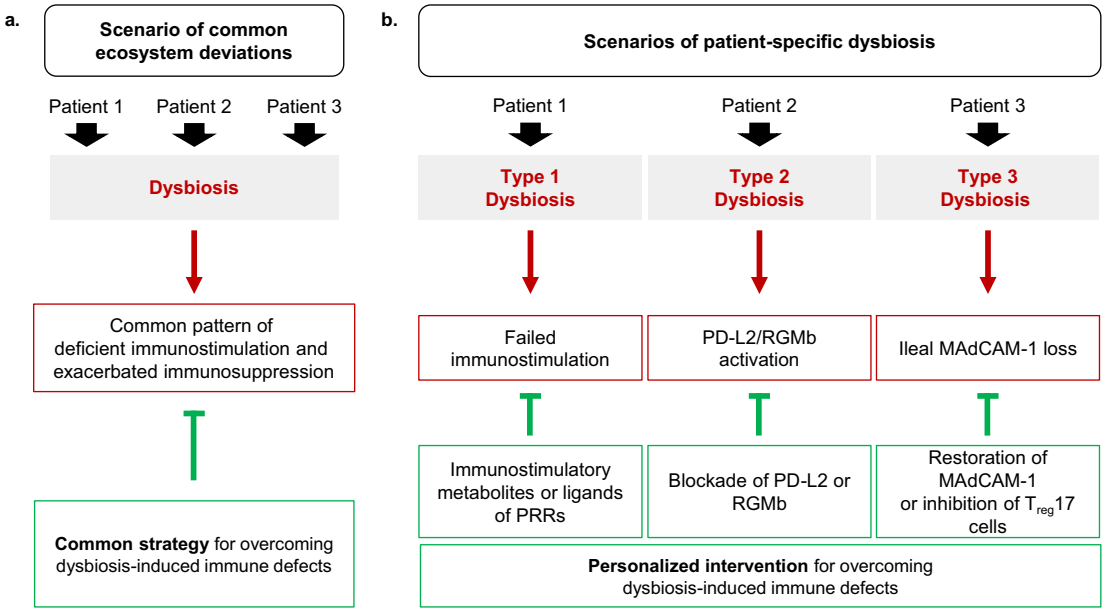

Figure 1. Scenarios of dysbiosis-induced immune failure. (a) Scenario of a deviated ecosystem in which multiple intersecting and hierarchically interconnected alterations explain the failing anticancer immune response as an ecosystem. (b) Scenario of patient-specific mechanisms in which different types of dysbiosis require personalized intervention to restore the anticancer immune response. PRRs: pattern recognition receptors.

note, RGMb was found to be cell-surface expressed on tumor-infiltrating $CD8^+$ T cells only from germ-free mice as compared to SPF mice. Moreover, conditional knockout of RGMb in T cells (but not in macrophages) was sufficient to restore the efficacy of PD-L1 blockade against MC38 cancers implanted into germ-free mice[5].

Altogether, the aforementioned data can be interpreted to mean that dysbiosis subverts anticancer immune responses elicited by blockade of the PD-L1/PD−1 interaction through the upregulation of an alternative pair of molecules that together constitute an immune checkpoint, namely PD-L2 and RGMb. PD-L2 is known to be mostly expressed by myeloid cells and their derivatives (such as Langerhans cells and microglia), while RGMb is widely expressed in multiple cell types (https://www.proteinatlas.org/). However, the functional role of these molecules in failing anticancer immune responses appears to be restricted to DCs in lymph nodes (but not in tumors) and tumor-infiltrating T cells. It is on these cell types that PD-L2 and RGMb are upregulated in dysbiosis, and adoptive transfer of PD-L2 knockout DCs as well as conditional knockout of RGMb in T lymphocytes alone is sufficient to improve anticancer immunosurveillance in the context of dysbiosis to enhance the therapeutic response of tumors to PD-L1 blockade.

Nonetheless, it appears that the combination of PD-L1 and PD-L2 blockade is more efficient against various cancers than PD-L1 blockade alone, in specific pathogen-free mice bearing B16 melanomas or Py8119 mammary tumors expressing ovalbumin[5]. There are two possible interpretations of this finding. First, it is possible that joint PD-L1 and PD-L2 coblockade is more efficient when standalone PD-L1 blockade is insufficient to stimulate tumor growth-controlling immune responses, irrespective of the absence or presence of dysbiosis (and hence the upregulation of PD-L2 and RGMb). Second, given that some but not all tumors are able to rapidly (within a week after their subcutaneous implantation), induce a state of dysbiosis[6], it is also plausible that the combined PD-L1/PD-L2 blockade is more efficient than PD−1/PD-L1 blockade against such tumors because of the presence of a dysbiotic state activating the PD-L2/RGMb checkpoint. This possibility requires urgent experimental clarification.

In addition, it will be interesting to weight the importance of dysbiosis-induced activation of the PD-L2-RGMb immune checkpoint[5] against that of other reported mechanisms such as the depletion of immunostimulatory microbial metabolites[7] and pattern recognition receptor agonists[8,9], or the dysbiosis-induced downregulation of ileal MAdCAM−1 that favors the exodus of immunosuppressive T cells from the gut-associated lymphoid tissue toward the tumor bed and tumor-draining lymph nodes[10]. Comparison with this latter mechanism is very attractive because in their paper, Park *et al.* proposed that PD-L2 suppression on DCs could originate in mLNs before migrating to tumor dLNs. It will be interesting to learn whether such mechanisms may co-exist and cooperate in the same setting (i.e., in the same mouse model or the same oncological patient) or whether, on the contrary, different types of dysbiosis exist in distinct models and individuals, requiring the personalization of dysbiosis-circumventing immunotherapies (Figure 1).

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Disclosure of potential conflicts of interest

LZ has held research contracts with Glaxo Smyth Kline, Incyte, Lytix, Kaleido, Innovate Pharma, Daiichi Sankyo, Pilege, Merus, Transgene, 9 m, Tusk and Roche, was on the on the Board of Directors of Transgene, is a cofounder of EverImmune, and holds patents covering the treatment of cancer and the therapeutic manipulation of the microbiota. GK has been holding research contracts with Daiichi Sankyo, Eleor, Kaleido, Lytix Pharma, PharmaMar, Osasuna Therapeutics, Samsara Therapeutics, Sanofi, Sotio, Tollys, Vascage, and Vasculox/Tioma. GK has been consulting with Reithera. GK is on the Board of Directors of the Bristol Myers Squibb Foundation France. GK is a scientific co-founder of EverImmune, Osasuna Therapeutics, Samsara Therapeutics, and Therafast Bio. GK is the inventor of patents covering therapeutic targeting of aging, cancer, cystic fibrosis, and metabolic disorders. GK's brother, Romano Kroemer, was an employee of Sanofi and now consults for Boehringer-Ingelheim. The funders had no role in the design of the study, in the writing of the manuscript, or in the decision to publish the results.

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Data availability statement

All data that led to the conclusions in this manuscript have been referenced and all sources have been described.

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