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FOCUSSED RESEARCH REVIEW

CD4$^+$ T cells indirectly kill tumor cells via induction of cytotoxic macrophages in mouse models

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Abstract

It is well recognized that CD4$^+$ T cells may play an important role in immunosurveillance and immunotherapy against cancer. However, the details of how these cells recognize and eliminate the tumor cells remain incompletely understood. For the past 25 years, we have focused on how CD4$^+$ T cells reject multiple myeloma cells in a murine model (MOPC315). In our experimental system, the secreted tumor-specific antigen is taken up by tumor-infiltrating macrophages that process it and present a neoepitope [a V region-derived idiotypic (Id) peptide] on MHC class II molecules to Th1 cells. Stimulated Th1 cells produce IFNγ, which activates macrophages in a manner that elicits an M1-like, tumoricidal phenotype. Through an inducible nitric oxide synthetase (iNOS)-dependent mechanism, the M1 macrophages secrete nitric oxide (NO) that diffuses into neighboring tumor cells. Inside the tumor cells, NO-derived reactive nitrogen species, including peroxynitrite, causes nitrosylation of proteins and triggers apoptosis by the intrinsic apoptotic pathway. This mode of indirect tumor recognition by CD4$^+$ T cells operates independently of MHC class II expression on cancer cells. However, secretion of the tumor-specific antigen, and uptake and MHCII presentation on macrophages, is required for rejection. Similar mechanisms can also be observed in a B-lymphoma model and in the unrelated B16 melanoma model. Our findings reveal a novel mechanism by which CD4$^+$ T cells kill tumor cells indirectly via induction of intratumoral cytotoxic macrophages. The data suggest that induction of M1 polarization of tumor-infiltrating macrophages, by CD4$^+$ T cells or through other means, could serve as an immunotherapeutic strategy.

Keywords Immunotherapy · Immunosurveillance · CD4$^+$ T cells · Macrophages · iNOS · Multiple myeloma

Abbreviations

CDR3	Complementarity-determining region
Id	Idiotype
iNOS	inducible nitric oxide synthase
L chain	Light chain
NO	Nitric oxide
Trp1	Tyrosinase-related protein 1

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Early experiments pointing to a role of CD4$^+$ T cells in tumor rejection

The notion that CD4$^+$ T cells can be important for rejection of cancer in humans is supported by a growing number of studies [1–4]. Initial evidence of the importance of CD4$^+$ T cells in rejection of tumor cells was provided already by more than 30 years ago in mouse studies [5, 6]. However, these pioneering studies neither defined the specificity of the tumor-eradicating CD4$^+$ T cells, nor the mechanism by which tumor cells were killed. During the same time period, antigen-specific CD4$^+$ T-cell clones were demonstrated to be cytotoxic in vitro [7, 8]. A few years later, cloned CD4$^+$ T cells were shown to reject B-lymphoma [9] and myeloma cells [10] in vivo in Winn-type assays where tumor cells and T cells were co-injected subcutaneously (s.c.), a rather artificial experimental system.

The tumor model (MOPC315) used by us to demonstrate tumor rejection by CD4$^+$ T cells

The successful generation of T-cell receptor (TCR) transgenic mice allowed for more physiologically relevant and definitive experiments addressing the tumor-protective role of CD4$^+$ T cells. In 1992, we established a TCR-transgenic model where the TCR recognizes a myeloma protein V region-derived peptide presented on an MHC class II molecule [11]. The model is based on the well-studied MOPC315 multiple myeloma model [12]. The MOPC315 tumor cells produce an IgA myeloma protein with λ2 light (L) chains (Fig. 1a). The particular λ2 L chain, referred to as λ2^{315}, expresses several mutations specific for the MOPC315 tumor cells (Fig. 1b, c). The starting point for generation of TCR-transgenic mice was a CD4$^+$ T cell clone, established in 1986, with specificity against residues 91–101 of the λ2^{315} sequence (Fig. 1d). This complementarity-determining region (CDR)3 sequence contains three mutated residues in position 94, 95 and 96 that were shown to be crucial for T-cell recognition [7, 13, 14]. The 91–101 CDR3 sequence spans the V–J junction and thus depends on a Vλ2→Jλ2 gene segment rearrangement that presumably occurred during the development of the progenitor B cell that finally gave rise to malignantly transformed MOPC315 cells (Fig. 1b). Later, the progenitor B cell most likely underwent a germinal center (GC) reaction where it acquired the somatic mutations in the CDR3 of its λ2 chain, prior to becoming malignantly transformed (Fig. 1c). Thus, the V region-derived sequence used in our tumor immunological studies represents a neoepitope unique to MOPC315 multiple myeloma cells. Consistent with a tradition of using the term idiotype (Id) to describe the antigenic determinants in Ig V regions, the MOPC315 antigen recognized by the TCR-transgenic CD4$^+$ T cells is referred to as an idiotypic (Id) peptide. The Id^{315} peptide is presented on the I-E^d MHC class II molecule expressed in BALB/c mice. The Id-specific TCR-transgenic mouse was established by transfer of TCR α and β genes from an Id-specific CD4$^+$ Th1 clone (Fig. 1d) [11].

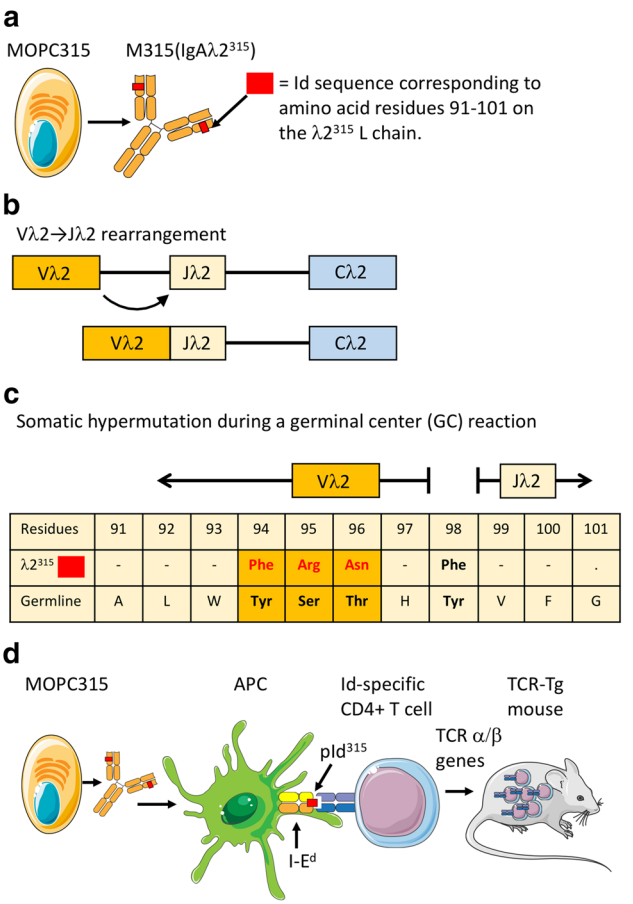

Fig. 1 The MOPC315 multiple myeloma cell line, the tumor-specific antigen, and the tumor-specific TCR-transgenic mice. **a** The BALB/c MOPC315 cell line [12] secretes an IgA myeloma protein with a λ2 L chain that expresses an Id sequence in complementary determining region 3 (CDR3) (red rectangles). **b** The B-cell progenitor that gave rise to the MOPC315 myeloma presumably underwent a Vλ2→Jλ2 rearrangement during its development in the bone marrow. Figure is simplified. **c** After exit to the periphery, the B cell presumably underwent a germinal center reaction resulting in acquired somatic mutations in codons 94, 95 and 96 [48] in its λ2 L chain, hereafter called λ2^{315}. Residues 91–101 of λ2^{315} represent an idiotypic (Id) peptide that fulfills the criteria of a tumor-specific neoantigen. Mutated residues are indicated in red. The 94–96 triplet is indicated in orange. The Vλ2- and Jλ2-encoded residues are indicated by arrows. Junctional residue 98 is in bold; in this position Tyr is more frequently observed than Phe. **d** M315 is endocytosed and processed by antigen presenting cells (APC) and the Id peptide (pId^{315}) is presented on the MHC class II molecule I-E^d to cloned Id-specific CD4$^+$ T cells [7, 13, 21]. TCR α and β genes were isolated from an Id-specific CD4$^+$ T cell clone and used to establish an Id-specific TCR-transgenic mouse on a BALB/c background [11]. The figure was made with components from Servier Medical Art (https://smart.servier.com)

Id-specific TCR-transgenic mice reject MOPC315 multiple myeloma cells

In 1994, we demonstrated that Id-specific TCR-transgenic mice on a BALB/c background were protected against subcutaneous tumor challenge with MOPC315 cells, but not an unrelated BALB/c-derived multiple myeloma cell line, J558 [15] (see Fig. 2a). Normal mice succumbed to both tumors. Transfer experiments demonstrated that about 4×10^5 CD4$^+$ T cells were required for tumor protection. Similar results were obtained with A20 B-lymphoma cells transfected with the λ2^{315} gene. Tumor eradication resulted in a conversion of naive Id-specific T cells for the most part into Th1 cells [15].

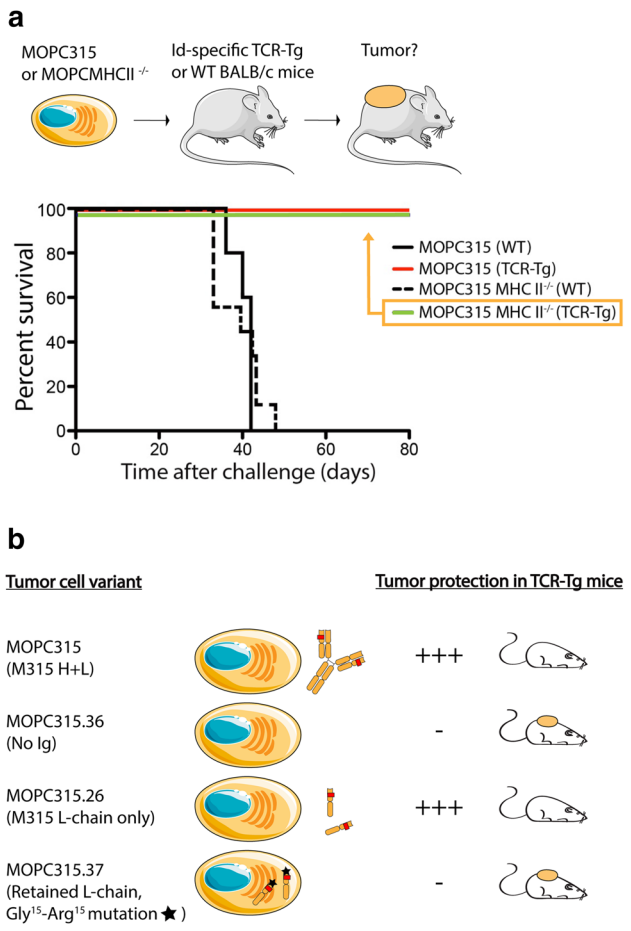

Fig. 2 Id-specific TCR-transgenic mice are protected against a challenge with MOPC315 cells. Protection does not require MHCII expression on MOPC315 cells, while secretion of myeloma protein is needed. **a** Idiotype (Id)-specific TCR-transgenic mice were protected against MOPC315, while WT BALB/c mice were not [15]. MOPC315 cells ablated for MHC class II I-E^d expression ($MHCII^{-/-}$) were still rejected by Id-specific TCR-transgenic mice [20]. Protection depended on Id-specific $CD4^+$ T cells, while CD8 cells or B cells were not needed since protection was observed in recombination-deficient TCR-transgenic SCID mice [16]. **b** MOPC315 cells that secrete the complete M315 myeloma protein and MOPC315.26 cells that only secrete the free $\lambda 2^{315}$ chains were both rejected by TCR-transgenic mice. MOPC315.37 variant cells that retain $\lambda 2^{315}$ chains intracellularly due to a $Glycine^{15} \rightarrow Arginine^{15}$ mutation were not rejected. Hence, tumor cell rejection requires secretion of the tumor-specific antigen [28]. The figure was made with components from Servier Medical Art (https://smart.servier.com)

The above experiments did not rule out the contribution of $CD8^+$ T cells or B cells to tumor rejection. The tumor challenge experiments were therefore repeated in TCR-transgenic mice made recombination deficient due to homozygous introduction of the *scid* mutation [16]. Such TCR-transgenic SCID mice lack $CD8^+$ T cells and B cells, and their Id-specific $CD4^+$ T cells do not express endogenous TCR chains. Nevertheless, TCR-transgenic SCID mice were protected against MOPC315, demonstrating that Id-specific $CD4^+$ T cells sufficed for protection, and that other parts of adaptive immunity such as $CD8^+$ T cells and B cells were dispensable [16]. To our knowledge, these are the first reports [15, 16] demonstrating that $CD4^+$ T cells with specificity for a neoantigen can reject tumor cells.

Tumor cells do not need to express MHC class II molecules to be rejected

In 1993, it was found that MOPC315 cells, similar to most multiple myeloma cells, lack expression of MHC class II molecules [10]. Consistent with this, cloned Id-specific $CD4^+$ T cells could not directly kill MOPC315 cells in vitro. However, when macrophages expressing the relevant MHC class II molecule were added to cultures, Id-specific $CD4^+$ T cells could initiate killing of MOPC315 cells. Macrophages had no effect if they lacked the relevant MHC class II molecule. These experiments represented the first indication that $CD4^+$ T cells can kill $MHCII^{NEG}$ tumor cells indirectly via macrophages, at least under in vitro conditions.

Conceivably, MOPC315 tumor cells could start to express MHC class II molecules in vivo, explaining why TCR-transgenic mice rejected MOPC315 cells. However, despite extensive efforts, we were unable to demonstrate induction of MHC class II molecules on MOPC315 cells in vivo [10, 17, 18]. Nevertheless, we later showed that it is possible to artificially induce MHC class II expression on MOPC315 cells by forced overexpression of Class II Major Histocompatibility Complex Transactivator (CIITA) [19], an essential co-activator of MHC II transcription, which is normally epigenetically silenced in this cell line. Therefore, it remained theoretically possible that MHCII expression could be present in some MOPC315 cells during in vivo growth. To conclusively exclude a requirement for tumor cell-intrinsic MHCII expression, we knocked out I-E^d expression by CRISPR/Cas9-mediated ablation of the alpha- or beta chain-encoding genes. Such MOPC315 cells, deficient in I-E^d, were still rejected by TCR-transgenic mice, conclusively demonstrating that MHCII expression on tumor cells is not required for tumor rejection by Id-specific $CD4^+$ T cells [19, 20] (Fig. 2a).

We later extended these studies to A20 BALB/c B-lymphoma cells that constitutively express a high amount of MHC class II molecules. A20 cells that had been transfected with the $\lambda 2^{315}$ gene (F9 cells) constitutively presented the pId^{315}: I-E^d to Id-specific $CD4^+$ T cells [21, 22]. Moreover, the F9 cells were rejected by Id-specific TCR-transgenic mice [15]. When I-E^d expression in F9 cells was removed by CRISPR/Cas9-technology, B-lymphoma cells were still rejected, confirming the results with the MOPC315 cell line [23].

In an effort to test the generalization of the above finding to tumors of non-B-cell origin, we performed experiments using the well-characterized B16 melanoma cell line. Several studies have demonstrated that palpable B16 melanoma tumors are rejected by treatment with a combination of sublethal irradiation, anti-CTLA4 mAb and adoptive transfer of TCR-transgenic $CD4^+$ cells specific for the tyrosinase-related protein 1 (Trp1) antigen presented in the MHCII I-A^b molecule [24]. The B16 tumor cells are MHC class II^{NEG} in vitro, but MHC class II expression has been observed following in vivo growth, presumably through effects of locally secreted factors such as IFNγ [25, 26]. It has further been shown that rejection of B16 tumor cells by this regimen is also effective in MHCII-deficient recipients, confirming that direct, cytotoxic killing of MHCII-expressing tumor cells is sufficient for therapeutic responses in this model [25]. However, it remained possible that indirect responses involving intratumoral macrophages might still be operational, and thus provide a level of redundancy. Indeed, when we ablated I-A^b-encoding genes in B16 cells by CRISPR/Cas9-technology, tumor rejection could still be observed following the same regimen of adoptive transfer of Trp1-specific $CD4^+$ T cells [23]. Similar results were also reported by another group looking at recognition of an ectopically expressed model antigen that could not be presented by the B16 tumor cells themselves [27].

Collectively, these studies demonstrate that tumor cell-intrinsic expression of MHC class II molecules is not required for $CD4^+$ T-cell-mediated killing. However, the findings also raise questions about the identity of the antigen presenting cells (APC) that present tumor-specific antigen to the $CD4^+$ T cells and how these APC become primed with the tumor-specific antigen. We have addressed these questions in the MOPC315 model (see below).

$CD4^+$ T-cell-mediated tumor rejection is dependent on secretion of tumor-specific antigens

By using variants of MOPC315 cells that either secrete or retain the myeloma protein intracellularly, we demonstrated that secretion was required for tumor rejection in TCR-transgenic mice [28] (Fig. 2b). Thus, a variant that retains the $\lambda2^{315}$ chain intracellularly, due to a $Glycine^{15} \rightarrow Arginine^{15}$ mutation, was not rejected. Consistent with this, in mice with tumors formed by this variant, we observed only negligible pId:MHCII priming of dendritic cells in draining LN, and of macrophages infiltrating the tumor [28].

Myeloma cells secrete a large amount of myeloma protein, and we therefore extended the studies to F9, a B-lymphoma model with a substantially lower level of antigen secretion. F9 is derived from the A20 lymphoma cell line (IgG2aκ), and was generated by transfection with the $\lambda2^{315}$-encoding gene, or mutated or truncated variants of this gene [21, 22]. Non-secreting transfectants were generated by introducing the lysine–aspartic acid–glutamic acid–leucine (KDEL) endoplasmic reticulum retention motif. We found that also in this setting, there was requirement for antigen secretion. While transfectants that secreted the $\lambda2^{315}$ chain above a certain level were effectively rejected by TCR-transgenic mice [15], transfectants that retained the $\lambda2^{315}$ chain intracellularly were not rejected [15].

It should be noted that these B-lymphoma variants, even though the Id protein was retained in the secretory pathway, were still able stimulate Id-specific Th1 cells in vitro [15, 22]. Thus, display of pId:MHCII on host APC appears to be required for tumor rejection to occur, while display of pId:MHCII only on tumor cells themselves is not sufficient. Consistent with this, secretion of the tumor-specific antigen was necessary and sufficient for rejection when the relevant MHC II molecule (I-E^d) was deleted in various B-lymphoma variants (see above) [23].

Immunoglobulins are highly secreted tumor-specific antigens, a feature that may be relatively unique to B-cell malignancies. An obvious next step was therefore to ask whether similar modes of indirect antigen recognition might be relevant in other types of malignancies. The secretion status of most tumor antigens remains poorly characterized, although there appears to be a general assumption that most are poorly secreted. To our surprise, we found that the Trp1 antigen in B16 cells is in fact secreted [23], consistent with findings in a previous publication [29]. This fits well with the observation that if I-A^b is deleted in B16 cells, the cells can still be rejected by Trp1-specific $CD4^+$ T cells [23], indicating that presentation of Trp1 on I-A^b on tumor cells is dispensable for tumor rejection by Trp1-specific $CD4^+$ T cells. However, a demonstration that lymph node dendritic cells and tumor-infiltrating macrophages present Trp1:I-A^b is lacking. Clearly, the issue of uptake of tumor-specific antigens by host APC, either by secretion or other mechanisms, should be more broadly addressed, in particular in the context of therapeutically relevant $CD4^+$ T-cell responses. Studies of the occurrence of secretion of tumor-specific antigens, and their presentation by host APCs might be facilitated by recent high-throughput proteomics and ligandomics technologies.

Tumor-infiltrating macrophages present tumor-specific antigen on their MHC class II

To identify the APC responsible for T-cell priming and subsequent intratumoral engagement in the course of tumor rejection, we examined APCs in the tumor and in the tumor-draining lymph nodes. Large MOPC315 tumors were

established in BALB/c mice; such tumors contained APC with dendritic cell and macrophage markers that stimulated Id-specific CD4$^+$ T cells in vitro [18, 30]. For this to occur, the tumor-infiltrating APC had to express the relevant MHC class II molecule (I-E^d) [18].

Next, to extend these experiments to MOPC315 tumors infiltrated by Id-specific CD4$^+$ T cells, we adopted a strategy to induce tumors in TCR-transgenic mice that normally reject inoculated MOPC315 cells. This was achieved by injecting TCR-transgenic mice with an increased amount of MOPC315 cells, an approach that partly overcomes effective tumor immunosurveillance, resulting in eventual tumor outgrowth in the majority of the mice [31, 32]. MOPC315 tumors of small sizes were studied to avoid the issue of inducible peripheral tolerance of Id-specific CD4$^+$ T cells observed in large tumors [31]. These studies revealed that small MOPC315 tumors are extensively infiltrated with APCs with dendritic cell and macrophage characteristics. Proliferating Id-specific CD4$^+$ T cells were also present in the tumors, although in lower numbers [18].

Macrophages are essential in early tumor cell rejection mediated by CD4$^+$ T cells

In the above studies, established tumors were investigated [18, 28]. To study very early events in tumor rejection, prior to any T-cell tolerance development, we developed a method in which MOPC315 tumor cells suspended in a Matrigel solution were injected into TCR-transgenic mice. The injected Matrigel solidifies in vivo subsequent to s.c. injection, and the resulting gel plug contains islets of proliferating tumor cells. Important to this experimental strategy, host-derived cells penetrate into the gel plug. The Matrigel can be dissected out at early time points, and its cellular contents analyzed by immunohistology and flow cytometry [17]. We found that macrophages infiltrated the MOPC315-containing Matrigel from very early on, detectable in significant numbers from around day 3. A few days later, around day 6, tumor-specific Th1 cells entered the tumor site, but in far lower numbers than macrophages. These tumor-infiltrating T cells had first, as naive T cells, been stimulated by dendritic cells that display pId:MHCII in the tumor-draining lymph node, attained a Th1 phenotype, and subsequently migrated to tumor [17, 18, 33, 34]. The tumor-infiltrating Th1 cells formed synapses with macrophages and activated them into M1-like cells through an IFNγ-dependent mechanism [17, 35]. The mutual interaction of Th1 cells and macrophages resulted in the elimination of MOPC315 cells around day 10–12 (Fig. 3a). The mechanism of tumor cell killing was proposed to occur through three phases: (1) Id priming of macrophages by secreted myeloma protein and stimulation of Th1 cells (2) IFNγ produced by Th1 cells activates

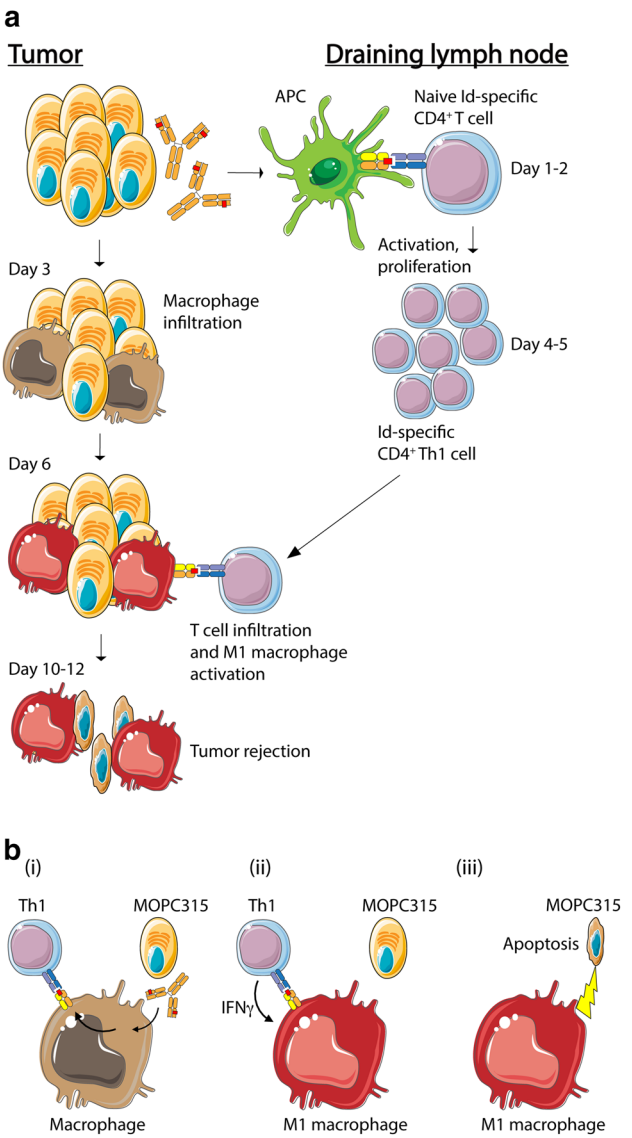

Fig. 3 The cellular mechanism by which CD4$^+$ T cells and tumor-infiltrating macrophages kill MOPC315 cells. Studies were facilitated by embedding MOPC315 cells in Matrigel, which solidifies in vivo, prior to s.c. injection into TCR-transgenic mice. The Matrigel plug can be dissected out and analyzed at any time point [17, 35]. **a** Cellular trafficking. Within 1–2 days after tumor cell injection, naive Id-specific CD4$^+$ T cells become activated by Id-primed dendritic cells in the draining lymph node [17, 18], proliferate, polarize into Th1 phenotype, and migrate to the tumor within day 6 [17, 33, 34]. In the meanwhile, the tumor is infiltrated by monocyte-derived macrophages that are Id primed and activate the Th1 cells, resulting in tumor eradication by day 10–12 [17, 18, 30, 35]. **b** Cellular interactions resulting in tumor rejection. (1) Myeloma protein secreted by MHCII^{NEG} MOPC315 cells is endocytosed and processed by tumor-infiltrating macrophages that in turn present pId^{315}:MHCII to Th1 cells. (2) Activated Th1 cells secrete IFNγ that induces M1 polarization of macrophages. (3) M1 macrophages kill MOPC315 myeloma cells [17, 35]. The figure was made with components from Servier Medical Art (https://smart.servier.com)

macrophages so that they attain an M1 phenotype (3) M1 macrophages kill MOPC315 myeloma cells (Fig. 3b) [17, 33]. The hypothesized molecular basis of how macrophages kill myeloma cells is outlined below.

The essential role of macrophages in CD4$^+$ T cell-mediated eradication of tumor cells was demonstrated in experiments where TCR-transgenic mice challenged with MOPC315 were treated with anti-chemokine (C–C motif) ligand 2 (CCL2) antibodies [36]. Such a treatment not only inhibited influx of macrophages into the tumor site, but also abrogated tumor rejection. This result indicates that the tumor-infiltrating macrophages are derived from monocytes, and that they are essential for tumor rejection. Further supporting a pivotal role, macrophages infiltrating MOPC315 tumors have been shown to display pId:MHCII as detected by a pId:MHCII-specific scFv fragment (unpublished data). Consistent with this, purified tumor-infiltrating macrophages stimulated Id-specific CD4$^+$ T cells in vitro [17, 18, 30].

The molecular mechanism by which tumor-infiltrating M1 MΦ kill tumor cells

The Th1/M1 macrophage mechanism does not result in tumor cell rejection in inducible nitric oxide synthetase (iNOS)-deficient mice (Fig. 4a) [36], consistent with observations of others that iNOS is required for tumor rejection [37]. It appears that IFNγ from Th1 cells induces M1 macrophages to upregulate iNOS, resulting in increased production and secretion of nitric oxide (NO). NO diffuses into tumor cells and react with oxygen radicals, resulting in the generation of high levels of peroxynitrite. The latter is apparent through increased nitrosylation of intracellular proteins, which eventually triggers tumor cell death by activation of the intrinsic apoptotic pathway [36]. In vitro, this form of killing appears to be effective up to a distance of about 100 µm from the macrophage (Fig. 4b) [36]. This spatial restriction might contribute to the escape of antigen-negative tumor cells from cytotoxic macrophages observed in experiments using tumors with heterogeneous antigen availability (see below).

Bystander killing by the Th1/M1 macrophage mechanism is limited, and variants that have lost expression of tumor-specific antigen escape rejection

Intuitively, one would expect that the Th1/M1 macrophage mechanism for rejection would result in elimination of tumor cells that had lost expression of antigen, simply because the indiscriminant nature of macrophage-mediated cytotoxicity should facilitate bystander killing of antigen-negative cells.

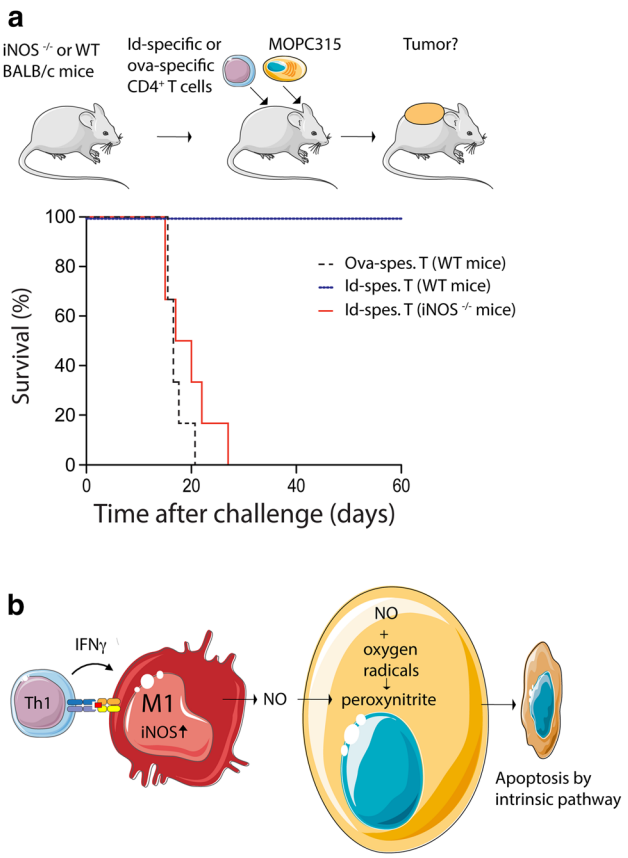

Fig. 4 The molecular mechanism by which tumor-infiltrating M1 macrophages kill MOPC315 cells. **a** Inducible nitric oxide synthetase (iNOS) is required for rejection. iNOS$^{-/-}$ or wild-type (WT) BALB/c mice were adoptively transferred with either Id- or ovalbumin (OVA)-specific CD4$^+$ T cells from TCR-transgenic mice, followed by s.c. injection of MOPC315 cells. Id-specific CD4$^+$ T cells protected WT mice but not iNOS$^{-/-}$ mice. The figure has been modified from [36]. **b** Id-specific Th1 cells secrete IFNγ that induces M1-like macrophages to produce NO. NO diffuses into tumor cells in the vicinity and reacts with oxygen radicals, resulting in generation of peroxynitrite, nitrosylation of proteins, and eventual apoptosis of the tumor cell. In vitro, NO-mediated killing is effective over distances up to about 100 µm [36]. The figure was made with components from Servier Medical Art (https://smart.servier.com)

However, such bystander killing is apparently not always sufficiently effective for elimination of a heterogeneous tumor. In our experiments, we consistently observed escape of antigen-negative tumor cells in experiments where antigen-positive and antigen-negative cells were mixed at various ratios (1:1 through 1:12) prior to implantation [38]. The basis for the observed, selective outgrowth of antigen-negative tumor cells in the face of macrophage-mediated killing remains unresolved, but could be related to the limited effective distance of M1 macrophage-mediated cytotoxicity. Moreover, we have observed that M1-polarized tumor-infiltrating macrophages appear to display limited movement throughout the tumor [38]. Through additional experiments, it was

demonstrated that MOPC315 myeloma cells escape rejection by down-modulation of $\lambda 2^{315}$ L chain secretion. This process results in outgrowth of cells with loss of secretion of free L chains, but with continued secretion of $\lambda 2^{315}$ assembled with heavy (H) chains as complete Ig molecules [39]. Our results show that free $\lambda 2^{315}$ chains are much more readily processed for MHC II presentation than the complete myeloma protein. Thus, free $\lambda 2^{315}$ chains yields more pId:MHCII complexes, and provides better stimulation of Id-specific $CD4^+$ T cells [7, 39]. In comparison, myeloma cells that only secrete stoichiometrically assembled H + L myeloma protein appear to be far less immunogenic. These studies emphasize that susceptibility of secreted tumor-specific antigen to antigen uptake and processing in macrophages is important for the efficacy of the indirect Th1/M1-mediated killing of tumor cells.

$CD4^+$ T cells can be used to treat cancers

The studies reviewed above employed surveillance-type experiments in TCR-transgenic mice. However, we have also found that adoptive transfer of $CD4^+$ T cells can be used to treat established B lymphomas [40] and multiple myeloma disseminated throughout the bone marrow compartment (Fig. 5) [19, 20, 41]. Both naïve $CD4^+$ T cells [20, 40], Th1 cells [20] and Th2 cells [42] have shown the ability to eliminate pre-existing tumors. A similar conclusion was reached in studies using the B16/Trp1-specific TCR-transgenic model; here, naïve Trp1-specific $CD4^+$ T cells appeared to be particularly efficient [25, 26].

Other mechanisms for $CD4^+$ T-cell-mediated killing of tumor cells

A number of other mechanisms for $CD4^+$ T-cell-mediated killing of tumor cells have been described. Th1 cells express Fas Ligand (FasL) enabling Th1 cells to kill B-lymphoma cells in vitro. However, in vivo, this mechanism was found to be dispensable, presumably because the Th1/M1macrophage mechanism can eliminate tumor cells in the absence of FasL [43]. $CD4^+$ T cells have been described to eliminate B16 melanoma cells through the acquisition of a cytotoxic phenotype involving perforin- and granzyme B-dependent killing [25, 26]. Hence, cytotoxic $CD4^+$ T cells clearly have the ability to directly eliminate MHC II-expressing tumor cells. Further experiments are warranted to determine the extent of induced MHC II expression on tumor cells in vivo, and the appearance of $CD4^+$ T cells with cytotoxic properties in other model systems. It has also been described that Th1 cells can secrete IFNγ and TNF, and thus induce senescence and death of

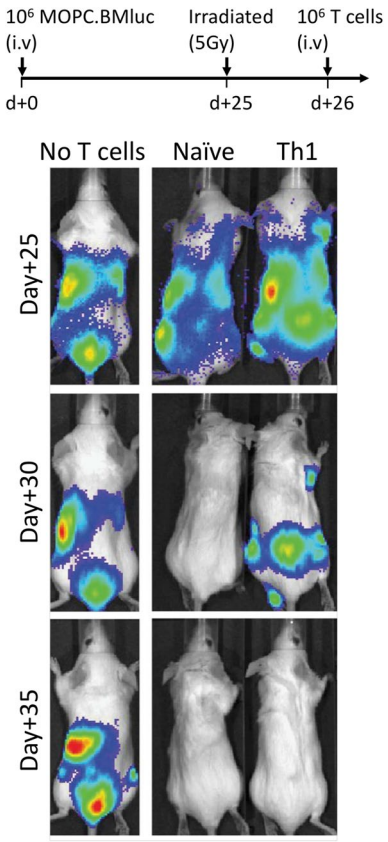

Fig. 5 Immunotherapy with $CD4^+$ T cells: transfer of T cells eradicates multiple myeloma disease in the bone marrow. Mice were injected i.v. with luciferase-expressing MOPC315.BM cells that have a propensity to home to the bone marrow [41]. On day 25, at which time point disease was evident in bone, spleen and liver; mice were sub-lethally irradiated and adoptively transferred with Id-specific $CD4^+$ T cells. Both naive $CD4^+$ T cells and Th1 cells induced curative anti-tumor immune responses. This figure has been modified from Fig. 2d in [20]

tumor cells [44]. $CD4^+$ T cells can interact not only with MΦ, but also with NK cells, resulting in tumor eradication [45]. The IFNγ produced by tumor-specific Th1 cells can interfere with tumor growth by destroying tumor vasculature [46]. Finally, $CD4^+$ T cells can support tumor-specific $CD8^+$ T cells, thus enhancing tumor eradication [47]. It is possible that activated, tumor-specific $CD4^+$ T cells might similarly act in synergy with other host cells within the tumor microenvironment to promote inhibition of tumor growth. A comprehensive insight into such processes could guide refinement of immunotherapeutic interventions, and possibly identify novel means of harnessing the immunomodulatory effects of $CD4^+$ T cells.

Author contributions BB wrote the paper with contributions from MF, OAH and AT. MF made the figures.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval and standards Animal experiments performed in the lab of Bjarne Bogen were approved by the Norwegian Animal Research Authority (Mattilsynet), and performed in accordance with institutional and Federation of European Laboratory Animal Science Associations (FELASA) guidelines.

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