



Th1 versus Th17: Are T cell cytokines relevant in multiple sclerosis?

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ABSTRACT

Our understanding of the pathophysiology of multiple sclerosis (MS) has evolved significantly over the past two decades as the fields of immunology and neurobiology provide new avenues of exploration into the cause and mechanism of the disease. It has been known for decades that T cells have different cytokine phenotypes, yet the cytokine phenotype of pathogenic T cells in MS is still an area of debate. In EAE, it appears that IFN γ and IL-17, produced by Th1 and Th17 cells respectively, are not the critical factor that determines T cell encephalitogenicity. However, there are molecules such as IL-23, T-bet and STAT4, that appear to be critical, yet it is unclear whether all these molecules contribute to a common, yet undefined pathway, or act in a synergistic manner which culminates in encephalitogenicity has still to be determined. Therefore, the focus of research on effector T cells in MS should focus on pathways upstream of the cytokines that define Th1 and Th17 cells, since downstream products, such as IFN γ and IL-17, probably are not critical determinants of whether an effector T cells is capable of trafficking to the CNS and inducing inflammatory demyelination.

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1. Introduction

Our understanding of the pathophysiology of multiple sclerosis (MS) has evolved significantly over the past two decades as the fields of immunology and neurobiology provide new avenues of exploration into the cause and mechanism of the disease.

Experimental autoimmune encephalomyelitis (EAE) has been used as a model for multiple sclerosis (MS) for more than 40 years and has been a major factor in determining the path of MS research. As the field of immunology advances, many of the fundamental observations in EAE are questioned and revisited to further our understanding of immune-mediated neurodegeneration with the ultimate goal of defining the pathophysiology of MS and development of a cure. Although MS is speculated to be a T cell-mediated autoimmune disease directed against myelin proteins, the cause of the disease is unknown. It has been known for decades that T cells have different cytokine phenotypes, yet the cytokine phenotype of pathogenic T cells in MS is still an area of debate. Understanding the phenotype of the T cells that mediate MS and other autoimmune diseases may be essential to determining the cause of disease and the development of therapies.

2. CD4 T cell cytokine phenotype in EAE

The EAE model evolved from the observation that a small number of individuals receiving the rabies virus vaccine, a live attenuated strain grown in rabbit CNS, developed encephalomyelitis. The realization that the encephalomyelitis was not caused by the rabies virus, but a hypersensitivity to the CNS debris contaminating the vaccine, initiated the development of EAE as a model for MS. Although EAE was initially induced by immunization with myelin proteins emulsified in Complete Freund's Adjuvant, it can also be induced by adoptive transfer of myelin-specific CD4⁺ T_H1 cells into naïve recipient mice [1–7]. Since myelin-specific CD4⁺ T_H1 cells were sufficient to induce EAE, MS research focused on these IFN γ -producing T cells in MS patients. Several studies that specifically suppressed IFN γ in the myelin-specific T cells prior to transfer into recipient mice demonstrated that altering the signaling pathway which results in IFN γ production in CD4⁺ T cells decreases the encephalitogenic capacity of these cells [6,8,9]. In addition, STAT4 and T-bet, which are transcription factors in the T_H1 differentiation pathway, have been shown to be essential for EAE induction [6,10–12]. The critical experiment to determine if IFN γ was in fact a viable target for autoimmune encephalomyelitis was the induction of EAE in IFN γ and IFN γ receptor deficient mice [13,14]. Not only were IFN γ -deficient mice susceptible to EAE, the disease appeared to be more severe. This observation was confirmed with systemic administration of antibodies to neutralize IFN γ [15,16]. The number of myelin-specific CD4⁺ T cells was expanded in IFN γ -deficient mice, which may have occurred due to loss of regulatory cells that were dependent on IFN γ .

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[17]. Together, these data suggest that the differentiation pathway that generates T_H1 cells may be important in encephalitogenicity, but the downstream production of $IFN\gamma$ by myelin-specific T cells is not critical.

The realization that $CD4^+$ T_H1 cells were sufficient to cause EAE, yet $IFN\gamma$ was not necessary, caused many investigators to look at other cytokines that may influence pathogenic capacity of T cells or focus on other characteristics of T cells such as signaling and transcription proteins. Since IL-12 was necessary for signaling events that promote the differentiation of T_H1 cells, mice deficient in the p40 and p35 proteins which comprise IL-12 were deleted in mice and EAE was evaluated. Although IL-12p40 deficient mice failed to develop EAE, the IL-12p35-deficient mice did develop EAE [18,19]. Since IL-12p40 was also a component of IL-23, the IL-23 specific protein p19 was deleted in mice and these mice were also resistant to EAE [20]. As a result, IL-23 was deemed an essential cytokine in EAE development, whereas IL-12 was not. In 2005, it was found that IL-23 could promote the expansion of myelin-specific IL-17+ T cells derived from MOG immunized mice and these IL-23-driven IL-17+ T cells were capable of transferring EAE [21]. This led to speculation that myelin-specific T_H17 cells were the primary encephalitogenic T cell population in EAE, and perhaps MS. Development of IL-17-deficient mice illustrated that although EAE was less severe in the absence of IL-17, IL-17 was also not necessary for the development of EAE [22,23]. Although IL-23 can enhance IL-17 production, the difference in susceptibility to EAE suggests that the essential role of IL-23 in EAE induction is not linked to its ability to promote IL-17 expression. The data from both the $IFN\gamma$ and IL-17 deficient mice suggested that cytokine production by myelin-specific T cells, although an easy potential therapeutic target, was not the determining factor for CD4 T cell encephalitogenicity.

Several studies found that IL-6 and $TGF\beta$ were sufficient to differentiate CD4 T cells into T_H17 cells in vitro [24–26]. However, T_H17 cells generated by differentiation with IL-6 and $TGF\beta$ in vitro are not encephalitogenic when transferred into naïve recipient mice, suggesting that this is not the mechanism that occurs in vivo to generate pathogenic T_H17 cells in EAE [27]. Recently, it has been demonstrated that $TGF\beta$ is dispensable during T_H17 differentiation, and that IL-6 in the absence of cytokines that promote T_H1 or T_H2 differentiation will generate T_H17 cells that are encephalitogenic [27,28]. Thus, it appears that T_H1 , T_H2 and T_H17 cells differentiate in distinct cytokine microenvironments which may be influenced by the host interactions with environmental factors such as pathogens.

3. Transcriptional regulation of CD4 T cell differentiation

Counter-intuitive data generated from mice deficient in transcription factors that play critical roles in T cell differentiation have also complicated attempts to define the pathogenic T cell population in EAE. Naïve CD4 T cells require both T cell receptor engagement with peptides presented by MHC class II molecules, as well as costimulation mediated by CD28 and B7 molecules. In addition, cytokines in the micro-environment influence the phenotype of CD4 T cells. T_H1 cells develop when the environment is rich in $IFN\gamma$ and IL-12 (Fig. 1A). $IFN\gamma$ binds to the constitutively expressed $IFN\gamma$ receptor ($IFN\gamma R$) on the surface of naïve CD4 T cells which results in phosphorylation of STAT1 (pSTAT1) and its translocation (Fig. 1C). pSTAT1 contributes to the transcription of T-bet which promotes the transcription of STAT1, and thus a positive feedback loop exists between these two transcription factors that initially drive a CD4 T cell towards a T_H1 phenotype. pSTAT1 and possibly T-bet transcriptionally activate the IL-12R β 2, which is the inducible chain of the IL-12 receptor and forms a heterodimer with the constitutively expressed IL-12R β 1 chain, forming a complete IL-12R on the cell surface. IL-12 in the micro-environment binds to the IL-12R, resulting in STAT4 phosphorylation, translocation and binding to the $IFN\gamma$ promoter. It has been demonstrated that pSTAT1, pSTAT4 and T-bet all bind to the $IFN\gamma$

promoter, as well as other transcription factors, and initiate $IFN\gamma$ production and contribute to the full differentiation of a T_H1 cell (Fig. 1C). Mice deficient in STAT4 and T-bet are resistant to EAE induction, suggesting that the T_H1 differentiation pathway is necessary for the generation of encephalitogenic CD4 T cells [6,10,11]. However, STAT1 mice are susceptible to EAE indicating the $IFN\gamma$ receptor signaling is not essential for disease induction [11].

ROR γ t has been identified as the essential transcriptional regulator of T_H17 cells. Mice deficient in ROR γ t have attenuated EAE [29]; however, these mice do not form lymph nodes [30]. Thus, the resistance to EAE may be due to the inability of CD4 T cells to be activated and differentiate in the periphery, and may be unrelated to a failure to generate T_H17 cells. In fact, T-bet deficient mice generate a high frequency of myelin-specific T_H17 cells upon EAE induction [27], yet fail to develop disease [11]; further indicating that myelin-specific T_H17 cells are not sufficient to cause EAE. Ex vivo analysis of myelin-specific T cells from mice immunized with MOG35–55 peptide indicates that T-bet is found in both T_H1 and T_H17 cells, suggesting that T-bet plays a role in T cell encephalitogenicity that is unrelated to the cytokine profile of the autoreactive T cells [27]. Generation of T_H17 cells with IL-6 in the absence of exogenous $TGF\beta$ generates T_H17 cells that express T-bet and can induce EAE when transferred into mice [27]. Although it is unclear what critical gene T-bet is regulating in encephalitogenic T cells, the IL-23 receptor and osteopontin genes have been shown to be regulated by T-bet [7,31]. Although osteopontin deficient mice have reduced EAE severity [32,33], IL-23 deficient mice are not susceptible to EAE [20], suggesting the IL-23 receptor gene may be the essential gene regulated by T-bet.

4. Role of cytokine phenotype on T cell trafficking and cell recruitment

One potential role of the cytokine phenotype is to mediate trafficking of myelin-specific T cells to distinct sites within the CNS which yield different functional deficits. Several studies have noted unique behavioral changes associated with myelin-specific T_H17 cells in EAE. In contrast to classical EAE induced by T_H1 cells in which mice develop an ascending paralysis, EAE induced by T_H17 cells is often characterized by spinning (dysequilibrium), ataxia, spasticity and proprioceptive defects [34]. Analysis of the distribution of T_H1 and T_H17 cells within the CNS revealed that infiltration of the brain parenchyma occurs when the number of T_H17 cells exceeds the number of T_H1 cells [34] (Fig. 1B). However, a wide range of T_H1 : T_H17 ratios permitted spinal cord parenchymal inflammation. This suggests that T_H17 cells may preferentially migrate to the brain, generating plaques at distinct sites with different functional losses. This was confirmed by administering an IL-17 neutralizing antibody to EAE-affected mice which resulted in a decrease in atypical EAE and an increase in classical EAE [34]. It should be noted that the absolute number of T_H1 cells infiltrating the CNS in EAE induced by immunization far exceeds the number of T_H17 cells, and lesions in the spinal cord are more prevalent early in the disease.

Adhesion molecules clearly contribute to EAE and MS has evidenced by the success of natalizumab, the anti-VLA4 antibody used to treat MS. In 1992, VLA4 was identified as the primary adhesion molecule mediating the attachment of lymphocytes to the CNS vascular endothelium [35]. It was subsequently observed that myelin-specific T_H1 cells that expressed VLA4 were highly encephalitogenic, while myelin-specific T_H1 cells that had minimal VLA4 expression were not encephalitogenic [36]. These two studies prompted the development of a humanized VLA4 antibody for the treatment of MS. Although natalizumab therapy has been complicated by rare cases of Progressive Multifocal Leukoencephalopathy [37], it has proven very effective in controlling CNS inflammation in MS patients [38]. Ex vivo analysis of VLA4 expression on CSF lymphocytes from MS patients illustrates that both T_H1 and T_H17 cells express high levels of VLA4

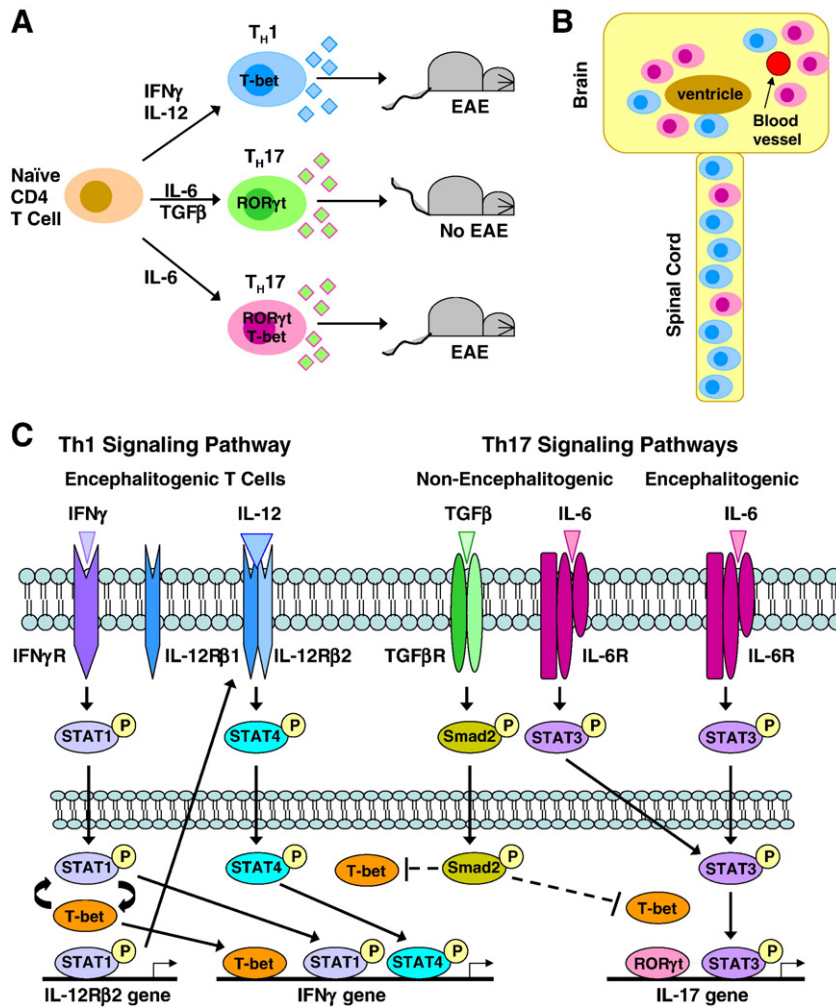


Fig. 1. Th1 and Th17 cell differentiation and trafficking to the CNS. (A) Naïve CD4 T cells can differentiate into Th1 or Th17 cells. During Th17 cell differentiation, TGF β can enhance IL-12 production, but this diminishes the capacity of these cells to induce EAE. (B) The distribution of Th1 and Th17 cells within the CNS varies in EAE. Th1 cells home preferentially to the spinal cord, while Th17 cells appear to preferentially traffic to the brain. The number of Th1 cells in the CNS significantly outnumbers Th17 cells in myelin-immunized mice. (C) Transcriptional regulation of Th1 and Th17 differentiation lends insight into signaling pathways that may be important in the development of encephalitogenic T cells. T-bet is found encephalitogenic Th1 and Th17 cells. The presence of TGF β during Th17 cell differentiation contributes to the inhibition of T-bet, resulting in non-encephalitogenic Th17 cells if primed with IL-6 + TGF β .

relative to Th1 and Th17 cells derived from the blood [39], suggesting that VLA4 expression plays a critical role in effector T cell entry into the CNS, regardless of cytokine phenotype.

Lesions in the spinal cords of mice immunized to induce EAE have high numbers of macrophages and activated microglia, a classic indicator of a Th1-driven inflammatory response. Consistent with this observation is the upregulation of ELR-CXC chemokines, CXCL9, CXCL10 and CXCL11, which are upregulated in the CNS of EAE-affected mice induced by transfer of Th1 cells [40]. In contrast, lesions induced by transfer of IL-23 driven Th17 cells are characterized by massive neutrophil recruitment and upregulation of the neutrophil-attracting ELR + CXC chemokines, CXCL1 and CXCL2 [40]. The critical role of IL-23 in the development of CNS inflammation was originally thought to be due to its role in enhancing IL-17 production, but more recently it has been found that IL-23 plays a critical role in homing to the CNS and survival of myelin-specific T cells in the CNS microenvironment [41]. This reconciles the data that IL-23 deficiency results in no disease, while IL-17 deficiency results in less severe EAE. Since loss of IL-23 prevents CNS inflammation there is no disease onset, while loss of IL-17 reduces neutrophil recruitment and secondary damage mediated by matrix metalloproteases. There has been speculation that IL-23 may influence T cell trafficking by modulating a chemokine receptor, such as CCR6 which has been

shown to be highly expressed on human Th17 [39,42]. However, there is contradictory data in CCR6 mice with regard to their EAE susceptibility [43–46], making it difficult to determine whether CCR6 is in fact a relevant player in the recruitment of Th17 cells to the brain. Interestingly, IL-23 driven Th17 cells also cause severe inflammation of the optic nerve, forming lesions reminiscent of neuromyelitis optica [40]. Together, these data suggest that the effector T cell cytokine phenotype may dictate the trafficking of the T cells to specific regions within the CNS, resulting in differential recruitment of innate immune cells and distinct functional losses.

5. Effector T cells in MS

Historically, myelin-specific T cells have been found in both MS patients and healthy individuals, which raised questions as to the relevance of these cells in MS patients. However, the observation that myelin-specific T cells from MS patients were more likely to have a Th1 phenotype gave validity to the hypothesis that these cells were potentially pathogenic in MS patients [47–50]. Subsequently, several studies demonstrated that although healthy individuals had myelin-specific T cells, these cells were naïve; whereas MS patients had activated and memory myelin-specific T cells, indicating that these cells had been previously activated in vivo [51–53]. In addition, a

clinical trial with an altered peptide ligand from myelin basic protein (MBP), which was intended to down-regulate myelin-specific T cells, actually exacerbated disease in several MS patients which was associated with increased frequency of MBP-specific T cells that produced IFN γ , suggesting that MS is mediated by myelin-specific T_H1 cells [54].

A decade ago it was found that IL-17 mRNA was augmented in the blood and CSF of MS patients, with IL-17 transcript levels higher in blood than CSF, as well as higher levels in blood during clinical exacerbations increased [55]. Subsequently, DNA microarray analysis of MS tissues indicated that IL-17 was present in MS lesions [56]. In 2005, a comprehensive analysis of cytokines expressed in the CSF of conventional MS and opticospinal MS was performed [57]. The Th1 cytokines, IFN γ and TNF α , were both higher in MS patients, but only the TNF α levels were statistically significant. However, comparison of number of CD4 T cells that expressed IFN γ was significantly higher the CSF lymphocytes relative to blood lymphocytes of MS patients, regardless of subtype of disease. In addition, the IFN γ /IL-4 ratio of CD4 T cells was significantly increased in conventional MS patients. IL-17 expression in the CSF of opticospinal MS patients was also significantly increased relative to controls and conventional MS. The spatial distribution of lymphocytes within the CNS of mice with EAE induced by IL-23 driven Th17 cells found extensive lymphocyte infiltration in the spinal cord from the subpial surface to deep within the white matter parenchyma, as well as extensive inflammation of the optic nerve, both characteristics of opticospinal MS [40].

An extensive comparison of Th1 and Th17 cells in the peripheral blood and CSF of MS patients found that the absolute number of Th1 cells both in the blood and CSF is about 10-fold higher than the number of Th17 cells [39], indicating that Th1 effector cells are significantly more prevalent both in the periphery and target organ in MS patients. However, there was a statistically significant increase in the number Th17 cells in the CSF during an exacerbation in MS patients that was not observed in the Th1 cells. This may be due to a role of Th17 cells in the exacerbation, but may also be due to the fact that the number of Th1 cells is already so much higher that changes may be more difficult to discern. IL-23 has been found to induce IL-17 expression in human memory CD4 T cells, with a significant proportion of these cells co-expressing IFN γ [58]. Although both Th1 and Th17 cells have been found capable of crossing the blood–brain barrier, the IFN γ + IL-17 + T cells in MS patients appear to cross the blood–brain barrier more efficiently, suggesting that this unique population of effector T cells may have greater encephalitogenic capacity [58].

Several clinical trials have been performed to determine if targeting effector T cells may be beneficial for MS patients. As early as the mid 1980s, the potential contribution of cytokines in regulating aberrant immune responses was recognized, prompting a clinical trial using IFN γ to treat MS patients which resulted in an increased number of exacerbations [59]. It was subsequently found that IFN γ induces apoptosis in human oligodendrocytes, and in MS lesions IFN γ expression colocalizes with apoptotic oligodendrocytes [60,61], indicating that IFN γ contributes to the pathology observed in MS lesions. These observations led to a clinical trial in which a neutralizing antibody specific for IFN γ was administered to MS patients, demonstrating a therapeutic benefit [62]. This trial was conducted at the same time as the EAE experiments in IFN γ -deficient mice and the anti-IFN γ treatment studies in EAE, which resulted in exacerbated disease [15,16]. The contradictory data between the human and mouse treatment studies with anti-IFN γ generated concern that inhibiting IFN γ may have unforeseen negative consequences. The role of IL-12/IL-23 pathways in mediating MS was recently tested in a phase II clinical trial in which MS patients were given a neutralizing antibody to the p40 subunit common to IL-12 and IL-23 [63]. In theory, this antibody

should prevent the differentiation of Th1 cells mediated by IL-12 and prevent the production of IL-17 mediated by IL-23. No significant changes in new gadolinium-enhancing lesions or clinical findings were observed. The negative outcome of this study does not necessarily mean that the IL-12/IL-23 is an inappropriate therapeutic target. The MS patient population was quite diverse in disease severity and duration, and since the IL-12/IL-23 pathway is critical during T cell differentiation, many of the patients may have established effector T cell populations that may mediate pathology independent of the IL-12/IL-23 pathway. In addition, it is unclear whether the antibody can and must access the CNS to neutralize T cells in the target organ.

IFN β , the most commonly prescribed immunomodulatory therapy for relapsing-remitting MS, has already been shown to mitigate effector T cells. IFN β therapies have been shown to dampen Th1 responses [64], but the ability of IFN β to attenuate Th17 responses is controversial. In vitro studies of human CD4 T cells found that IFN β inhibited Th17 cell differentiation and could induce apoptosis in Th17 cells [65,66]. However, an ex vivo analysis of IFN γ and IL-17 mRNA, as well as their respective transcription factors, T-bet and RORC, demonstrated that IFN β therapy for one year resulted in decreased IFN γ and T-bet levels but no significant changes in IL-17 or RORC expression [67]. Interestingly, decreases in T-bet mRNA levels were only associated with patients who had a clinical benefit from IFN β therapy, suggesting that T-bet expression may be a prognostic indicator of IFN β responsiveness in MS patients. This supports the hypothesis that T-bet is critical for T cell encephalitogenicity, irrespective of T cell cytokine phenotype.

6. Conclusion

Although the exact roles of Th1 and Th17 cells in the development of MS lesions are not established, it appears that both of these effector T cell populations can cause CNS inflammation and demyelinating lesions. The data in mice deficient in various cytokines, trafficking molecules and transcription factors indicate that encephalitogenic T cells have multiple critical molecules that give them the capacity to induce CNS inflammation. The observation that VLA4 high expressing Th1 cells are encephalitogenic, while VLA4 low expressing Th1 cells are not encephalitogenic illustrates that all Th1 cells are not equal in their pathogenic capacity [36]. Similarly, the observation that T-bet positive Th17 cells are encephalitogenic, while T-bet negative Th17 cells are not encephalitogenic demonstrates that Th17 cells differ in their pathogenic potential because of a transcription factor previously known for its role in Th1 cells [27]. The data in EAE does demonstrate that there are pathways mediated by T-bet and Stat 4 that are clearly important in encephalitogenicity [6,7,10,11,27]. In addition, IL-23, IL-1 β and IL-6 appear to contribute significantly to disease onset [20,21,27,68–70]. Whether these various transcription factors and cytokines all contribute to a common, yet undefined pathway, or act in a synergistic manner which culminates in encephalitogenicity has still to be determined. Analysis of the various molecules, previously shown to play a vital role in EAE, in MS patients has proven to be complicated and sometimes contrary to what has been observed in EAE. In particular, anti-IFN γ therapy was not beneficial in EAE, but showed promising results in a small clinical trial in MS [62]. However, VLA4 and T-bet expression appear to be involved in effector T cells in MS patients, similar to what has been shown in EAE. Therefore, the focus of research on effector T cells in MS should focus on pathways upstream of the cytokines that define Th1 and Th17 cells, since downstream products, such as IFN γ and IL-17, probably are not critical determinants of whether an effector T cell is capable of trafficking to the CNS and inducing inflammatory demyelination.

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