Expansion of CD28⁻CD27⁻NKG2D⁺ Effector Memory T cells and Predominant Th1-type Response during Febrile Attacks in Tumor Necrosis Factor-Associated Periodic Syndrome*

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Tumor necrosis factor-associated fever syndrome is an autosomal dominant disorder caused by mutations of the *TNFRSF 1A* gene encoding the 55 kD TNF receptor (p55 TNF-RI). Consequently, cleavage of the p55 TNF-R ectodomain upon cellular activation is impaired with diminished shedding of the potentially antagonistic soluble receptor. This results in dysregulated inflammatory responses and excess of TNFα and provides an *in vivo* model of the consequences of excess TNF. Studying the T cell phenotype of a patient suffering from TRAPS, we show an expansion of CD28⁻CD27⁻NKG2D⁺ effector memory T cells and a predominant Th-1 response during febrile attacks. Application of the human soluble p75 TNFα receptor fusion protein etanercept induced remission of all symptoms.

Patient Description

A 66 year old Caucasian woman was admitted to our department because of recurrent febrile attacks, oligoarthritis, myalgias and migratory erythematous macules since early adulthood. She had two sisters in Russia who were suffering from similar symptoms. Treatment with various immunosuppressants (gold, colchicine, methotrexate, azathioprine, leflunomide) was not effective.

Recurrent self-resolving fever and a positive family history was suspicious of a hereditary periodic fever syndrome. DNA analysis of the TNF receptor superfamily *(TNFRSF) 1A* gene disclosed a R92Q mutation. A skin biopsy showed small vessel vasculitis and panniculitis. Treatment with the TNFα-inhibiting human soluble p75 TNFα receptor fusion protein etanercept (2 x 25 mg/week subcutaneously) induced complete remission of all symptoms.

Comment

TRAPS is an autosomal dominant disorder characterized by self-limiting recurrent febrile acute attacks variably associated with serosal, synovial and/or cutaneous inflammation. A typical complication is AA-amyloidosis, which leads to nephritic syndrome and renal failure [1]. TRAPS is caused by mutations in the *TNFRSF* 1A gene encoding the 55 kDa TNF receptor (p55 TNF-RI) [1]. Several mutations have been found to disrupt conserved disulfide bonds. Impaired cleavage of the p55 TNF-R ectodomain upon cellular activation with diminished shedding of the potentially antagonistic soluble receptor results in dysregulated inflammatory responses and excess of TNFα. The R92Q mutation does not affect cysteine residues of the p55 TNF-R. Of note, receptor shedding also occurs in healthy individuals, but s(oluble) p55 TNF-RI levels fail to increase during febrile attacks in patients with the R92Q mutation [2].

TNFα down-regulates CD28 expression on CD4 T cells *in vitro* [3]. Moreover, several autoimmune disorders such as rheumatoid arthritis, ankylosing spondylitis and Wegener`s granulomatosis, in which TNFα plays an important pathophysiologic role, are characterized by the expansion of circulating effector memory T cells lacking co-stimulatory CD28 expression [4].

CD4⁻ and CD8⁻ T cell subsets were analyzed for CD28, CD27, NKG2D, HLA-DR and intracytoplasmic interferon-gamma, and interleukins 12, 10, 5, 4, and 2 expression by flow cytometric analysis. Four-color flow cytometric analysis was performed using a FACSCalibur™ flow cytometer and data acquired with CellQuest Pro™ software (BD, Heidelberg, Germany).TNFα, IFNγ, IL-10, IL-5, IL-4, and IL-2 cytokine production was determined during afebrile and febrile episodes using a cytometric bead array kit (BD, Heidelberg, Germany). Soluble p55 TNF receptor I (sTNF-RI) was quantified in serum by enzyme-linked immunosorbent assay (Quantikine, R&D Systems, Germany).

An expansion of $CD4^+CD28^-$ and $CD8^+CD28^-$ T cells was detected compared with healthy controls (n=5). About half of the $CD4^+CD28^-$ T cells also lacked co-stimulatory CD27 expression. The activating NKG2D receptor was strongly up-regulated and preferentially expressed on the expanded $CD4^+CD28^-$ T cell fraction. $CD8^+CD28^-$ T cells predominantly lacked CD27 expression. The phenotype of $CD8^+NKG2D^+$ T cells was significantly shifted towards the expanded $CD28^-$ cell fraction. HLA-DR (activation marker) expression was up-regulated both on $CD4^+$ and $CD8^+$ T cells with preferential expression on the $CD28^-$ T cell fractions [Figure]. $CD4^+$ and $CD8^+$ T cells displayed

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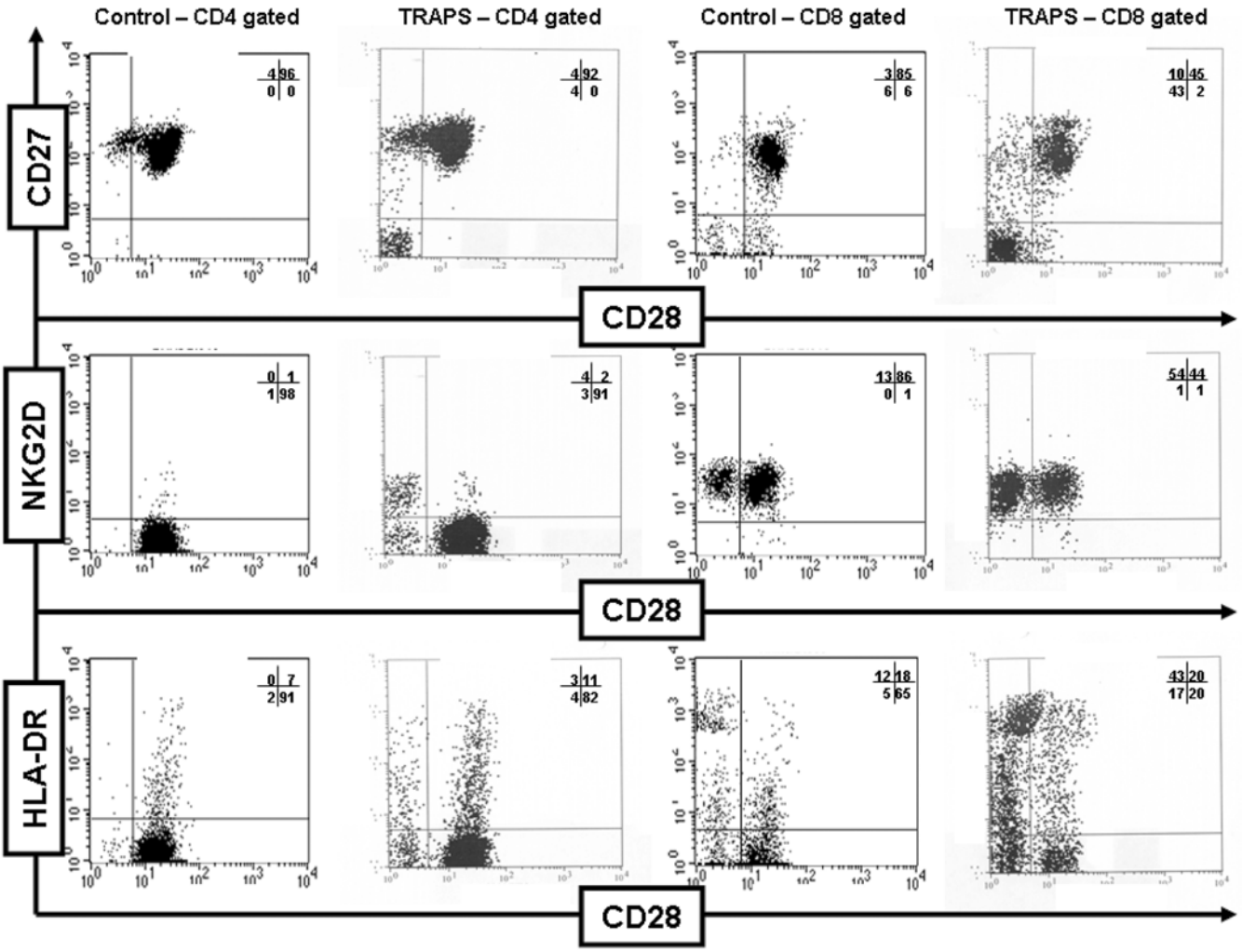
TNF = tumor necrosis factor
TRAPS = TNF-associated fever syndrome
IFNγ = interferon-gamma
IL = interleukin



CD28$^-$CD27$^-$NKG2D$^-$ effector memory T cells are expanded in TRAPS. T cells lacking CD28 expression are expanded in TRAPS as compared to healthy controls. Whereas CD27 expression is also down-regulated on many CD28$^-$ cells, anomalous expression of the activating NKG2D receptor is predominantly seen on the CD4$^-$CD28$^-$ T cell fraction. NKG2D is constitutively expressed on CD8$^-$ T cells. HLA-DR expression is also predominantly up-regulated on CD28$^-$ cells. The figure shows representative multicolor stainings of total CD4$^-$ and CD8$^-$ T cell populations. The dot plots show double staining for CD28/CD27, NKG2D/CD28, and HLA-DR/CD28.

low intracytoplasmatic IFNγ, IL-12, IL-10, IL-5, IL-4, and IL-2 expression during afebrile periods. Activation of CD4$^+$ and CD8$^+$ T cells with PMA and ionomycin resulted in prominent intracytoplasmic IFNγ, IL-12, IL-2, and IL-10 expression in both T cell subsets. Low production of TNFα, IFNγ, IL-10, IL-5, IL-4 and IL-2 was also detected in periods between fever attacks by CBA (TNFα, IFNγ, IL-5, IL-2: 0 pg/ml each; IL-10: 2.9 pg/ml, normal < 7.8 pg/ml; IL-4: 3.3 pg/ml, normal < 0.25 pg/ml). During febrile attacks there was a strong IFNγ response (113.9 pg/ml, normal < 15.6 pg/ml) and a less prominent TNFα (17.9 pg/ml, normal < 15.6 pg/ml), IL-10 (20.8 pg/ml) and IL-4 (39.9 pg/ml) response. Serial detection of the sTNF-RI with ELISA during afebrile and febrile periods disclosed values with slightly above the normal range (2183 and 2294 pg/ml respectively, normal 749–1966 pg/ml) with no apparent increase during febrile attacks (2171 pg/ml).

TNFα has been reported to down-regulate CD28 expression on CD4$^+$ T cells *in vitro* [3]. Since regulation of TNFα function is impaired in TRAPS, it provides a human *in vivo* model of biologic consequences of excess TNFα. We demonstrated expansion of CD28$^-$ cell fractions both within the CD4$^+$ and CD8$^+$ T cell compartments in TRAPS. The expanded CD28$^-$ T cell fraction is phenotypically reminiscent of so-called late differentiated or effector memory T cells, which often also lack CD27 expression. The activating NKG2D receptor is constitutively expressed on human CD8$^+$ T cells. It has been hypothesized that anomalous NKG2D expression on CD4$^+$CD28$^-$ effector memory T cells could facilitate autoimmune and chronic inflammatory reactions [5]. In line with other authors [2], we found no increases in soluble p55 TNF-RI levels during afebrile and febrile periods of the disease in this patient with a R92Q mutation. Instead, sTNF-

CBA = cytometric bead array
ELISA = enzyme-linked immunosorbent assay

RI levels were slightly above normal. Aksentijevich et al. [2] pointed out that the failure of sTNF-RI levels to increase with febrile episodes is suggestive of an *in vivo* functional abnormality of TNF-RI cleavage.

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