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がん・精巣抗原を標的としたATLに対する新規免疫療法の開発
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厚生労働科学研究費補助金（第３次対がん総合戦略研究事業）
総合研究報告書

「がん･精巣抗原を標的としたATLに対する新規免疫療法の開発」に関する研究

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研究要旨(まとめ)

本研究により、*ATL*患者のみならず、前癌状態といえる*HTLV-1*無症候性キャリアの段階で、がん・精巣抗原特異的免疫応答を獲得しているケースを認め、この免疫応答を増強させることにより*ATL*の発症予防法、あるいは*ATL*に対する新規治療法につながる可能性が示唆された。*2012*年に世界に先駆け日本で、再発又は難治性の*ATL*に承認された*CCR4*抗体*(*ポテリジオ®*)*は制御性*T*細胞*(Tregs)*のうち*effector Tregs* を特異的に除去する。この機序により*HTLV-1*関連抗原のみならず、*NY-ESO-1*など、がん精巣抗原に対する免疫応答が増強される可能性を示唆する結果を得た。すなわち、がん精巣抗原を標的とする免疫療法と*CCR4*抗体療法の併用療法は*ATL*の発症予防法、あるいは*ATL*に対する新規治療法として有望な可能性がある。

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A.　研究目的

*HTLV-1*感染者は日本国内に約*108*万人、世界には約*2,000*万人存在すると推定されており、現時点では*ATL*発症予防法は確立されていない。また、*ATL*は著しく予後不良な疾患であり、標準的治療は確立されていない。一方、*ATL*患者にみられる同種造血細胞移植療法での長期生存例は、本腫瘍に免疫療法が有効であることを示唆する。
研究者らは *ATL* に対する新規免疫療法の標的として、多くの固形がんに対する免疫療法の標的抗原として臨床試験が進んでいる、がん・精巣抗原を位置づけた。その上で、*HTLV-1/ATL* に対する新規治療開発研究*(CCR4* 抗体　モガムリズマブ*)*で実績を有する、石田、稲垣、宇都宮と、がん・精巣抗原に対する免疫応答分野で、日本を代表する研究者である西川が本研究班を構成した。その上で以下に示す *3* つの項目を研究目的に掲げた。

i) HTLV-1 感染から *ATL* 発症に至るまでの免疫病態の解明
*ii) HTLV-1*キャリアの*ATL*発症予防法の確立
*iii) ATL*に対するがん･精巣抗原を標的とした新規治療法の確立

B.　研究方法

ATL 患者、あるいは *HTLV-1* 無症候性キャリアから書面での説明の上、書面で同意を得て、末梢血単核球、血清、を取得した。末梢血単核球を用いて *HTLV-1* ウイルス量を測定するとともに、がん精巣抗原に対する特異的細胞免疫応答を解析した。血清を用いて *sIL2R* を測定するとともに *NY-ESO-1* と *XAGE-1b* に対する抗体価を測定した。リンパ節など生検試料を有する *ATL* 患者においては、同じく書面での説明と同意の上、試

料を取得。*NY-ESO-1* タンパク、*mRNA* の発現解析を実施、臨床情報、その他の免疫パラメーターと統合解析を実施した。本研究開始後の *2012* 年、研究者らが臨床開発に関わった *CCR4* 抗体*(*ポテリジオ*)*が、再発又は難治性の *ATL* に承認された。よって本研究の目的に

*iv) ATL*患者におけるがん･精巣抗原に対する免疫応答に、*CCR4*抗体が及ぼす影響の解明。

を加え、研究を遂行した。

C．研究結果

HTLV-1 キャリアにおいてがん･精巣抗原の発現様式、免疫応答を解析した。結果、複数の *HTLV-1* キャリアで *NY-ESO-1* に対する特異的 *T* 細胞応答を認めた。また *NY-ESO-1* に対する液性免疫応答を *11.1% (11/99)*に認めた。一方、*HTLV-1* 無症候性キャリア末梢血単核球の *RT-PCR* では *NY-ESO-1 mRNA* の検出は *95%*以上で陰性であった。
ATL 患者の生検標本において *NY-ESO-1* タンパクの発現は *40%*程度に認め、*NY-ESO-1 mRNA* の発現は *60%*程度に認めた。患者末梢血単核球の検討では *NY-ESO-1 mRNA* の発現は *25%*程度にとどまた。同一患者において、*NY-ESO-1* の発現は *ATL* の存在部位により異なった。*ATL* リンパ節での *NY-ESO-1* 発現は *mRNA*、タンパクともに予後への寄与はなかった。
ATL 患者では *NY-ESO-1* に対する液性免疫応答が *20.6% (14/68)*に認められた。次に、末梢血検体が十分に得られた *9* 名の *ATL* 患者において *NY-ESO-1* 特異的 *CD8* および *CD4+T* 細胞の同定を試みた。*CD8+T* 細胞誘導は、*HLA/*ペプチドテトラマー*(*テトラマーが使用可能な *HLA* を持っている患者*)*と *NY-ESO-1* 抗原刺激に対する細胞内サイトカイン染色法により検討した。*9* 症例中 *5* 症例で *NY-ESO-1* 特異的 *CD8+*T細胞応答が同定された。一方 *NY-ESO-1* 特異的 *CD4+*T細胞は *9* 症例中 *1* 症例で認めるのみであった。これは *CD4+* 細胞分画には *ATL* 細胞が残存しているためであると考えられた。*NY-ESO-1* 特異的 *CD8+*T細胞応答が同定された患者のうち *1* 症例で自己 *ATL* 細胞が得られたため、*NY-ESO-1* 特異的 *CD8+*T細胞が認識し、エフェクター機能を発揮できるかを検討した。*ATL* 患者由来 *NY-ESO-1* 特異的 *CD8+*T細胞は自己 *ATL* 細胞を認識し、*effector* サイトカインを産生した。

ヒトでは *FOXP3* 分子発現の *Tregs* 特異性が低いが、*FOXP3* の発現レベルと *CD45RA* により *FOXP3+CD4+*細胞を *3* つに分類することで、より厳密に *Treg* を定義することが可能である。すなわち、*naive Treg (CD45RA^{hi}FOXP3^{lo})*、*effector Treg (CD45RA^{lo}FOXP3^{hi})*、*non-Treg (CD45RA^{lo}FOXP3^{lo})*に分類され、*naive Treg* および *effector Treg* は免疫抑制活性を有するが、*non-Treg* は抑制活性を有しない。また、*naive Treg* より *effector Treg* の方が強い抑制活性を有する。*CCR4* の発現は *naive Treg* では認めず、*effector Treg* が強い。よって、*CCR4* 抗体で標的となる *Treg* は、理論上 *effector Treg* であるが、実際にモガムリズマブの治療を受けた *ATL* 患者では、*effector Treg* の存在比率は著しく低下し、*HTLV-1 Tax* に対する特異的 *CTL* は増加する傾向を認めた。また、*effector Treg* が低下した状態において *HTLV-1 Tax* 特異的 *CTL* の誘導効率は著しく向上した。このことは、モガムリズマブ治療によって *effector Treg* が除去されることにより、*HTLV-1 Tax* に対する免疫応答が増強することを意味している。
次に、抗 *CCR4* 抗体を用いた *effector Treg* 除去のがん・精巣抗原に対する免疫応答増強効果を検討した。がん・精巣抗原の中でも高頻度で *ATL* に発現が認められた *NY-ESO-1* に対する細胞性免疫応答の変化を検討した。健康人では、*CD4+T* 細胞から *NY-ESO-1* 特異的 *T* 細胞を誘導することが出来ないことが明らかになっている。そこで、健康人由来 *PBMCs* から *CCR4+effector Tregs* を除去し、*NY-ESO-1* 特異的 *CD4+T* 細胞誘導を検討したところ、*NY-ESO-1* 特異的 *CD4+T* 細胞誘導が *43.8%*で認められた。また、*NY-ESO-1* を発現しているが *NY-ESO-1* に対する免疫応答を惹起できない悪性黒色腫患者由来の *PBMCs* で同様のアッセイを行った。未処理の *CD4+T* 細胞からは *NY-ESO-1* 特異的 *CD4+T* 細胞が誘導できなかったが、*CCR4+*細胞を除去することで *NY-ESO-1* 特異的 *CD4+T* 細胞が *37.5%*の患者で誘導された。さらに、*NY-ESO-1* 特異的 *CD8+T* 細胞に対する影響を検討した。悪性黒色腫患者由来の *PBMCs* を用い、*NY-ESO-1* 特異的 *CD8+T* 細胞の誘導を試みたところ、*CCR4* 陽性細胞を除去することによって未処理よりも高頻度に *NY-ESO-1* 特異的 *CD8+T* 細胞が誘導された。以上より、抗 *CCR4* 抗体により *effector Tregs* を除去することにより、がん・精巣抗原特異的免疫応答が増強されることが示された。次に抗 *CCR4* 抗体の *in vivo* での効果を検討するため、抗 *CCR4* 抗体、モガムリズマブ投与前・

後の *ATL* 患者 *PBMCs* を採取し、*ATL* 細胞、*effector Tregs* に与える影響を検討した。モガムリズマブの治療を受けた *ATL* 患者では、*effector Treg* の存在比率は著しく低下し、*HTLV-1 Tax* に対する特異的 *CTL* は増加する傾向を認めた。一方、*NY-ESO-1* に対する免疫応答は、ウイルス抗原である *HTLV-1 Tax* に対する免疫応答に比較し、その程度が軽度であり、さらに症例間の変化が大きく、*CCR4* 抗体治療による、その動態の変化様式を結論するには至っていない。さらに、*HTLV-1 Tax* に対する液性、細胞性免疫反応の動態との相関を解析したが、統計学的に有意な相関を見出すに至っていない。しかしながら *CCR4* 抗体治療によって、細胞性、液性免疫ともに増強し、良好な臨床反応を得ている症例が存在している。このことは、*effector Treg* が除去されることにより、*NY-ESO-1* に対する免疫応答も、*HTLV-1 Tax* に対する免疫応答と同様、増強される可能性を示している。

D．考察

ATL は *HTLV-1* 感染から *50-60* 年の多段階発癌過程を有する。本研究で、*ATL* 発症前の *HTLV-1* 無症候性キャリアの段階、すなわち、いわば前癌病変の段階で、*HTLV-1* 感染細胞はがん・精巣抗原の発現を獲得しており、さらには特異的免疫応答が誘導されている一群が存在することが明らかになった。この一群の中には、がん・精巣抗原特異的免疫応答により、*HTLV-1* 感染細胞の増殖が抑えられ *ATL* にまで進展しない症例があると推察される。*HTLV-1* 無症候性キャリア、*ATL* ともに、末梢血単核球では *NY-ESO-1 mRNA* が検出困難であった。この理由として、抗原性を有した細胞 *(NY-ESO-1* 陽性細胞*)*が、宿主の抗腫瘍免疫が働きやすい環境*(*血液中*)*に出現しにくい可能性が考えられる。がん細胞は、リンパ節などがん微小環境内で宿主免疫反応から回避しつつ生存している。*HTLV-1* 感染細胞の体内での挙動を明らかにすることは今後の大きな課題である。

一般的に、*NY-ESO-1* をはじめとする、がん・精巣抗原の、がん細胞における機能は明らかではないが、固形がんにおいては病期の進行とともに発現を獲得し、その発現は予後不良因子になるとの報告が多い。しかしながら研究者らの解析では、*ATL* においては、*NY-ESO-1* の発現は予後不良因子ではなかった。*NY-ESO-1* 発現 *ATL* 細胞に対する宿主側の *NY-ESO-1* 特異免疫が、予後良好因子として作用している可能性が示唆され、更なる解析を必要とする。

*FOXP3*と*CD45RA*の発現レベルにより*FOXP3+CD4+*細胞を*3*つに分類することで、より厳密に*Treg*を定義することが可能な上、*CCR4*抗体の標的となる*Treg*分画が明らかになった意義は大きい。これまで、*Tregs*は抗腫瘍免疫応答を抑制しているため、全ての*Tregs*を除去する必要があると考えられてきたが、*CCR4*抗体で、*effector Tregs*のみを標的とすることで、ウイルス抗原、あるいはがん抗原、特異的免疫応答の増強につながる可能性が示唆された。さらには、*CCR4*抗体による *effector Tregs* 特異的除去では、*naive Tregs*は残存するため、*Tregs*本来の機能である自己に対する免疫応答をコントロールし、副作用軽減につながっている可能性がある。研究者らは、多施設共同前向き臨床研究”成人T細胞白血病リンパ腫に対するモガムリズマブ治療中の免疫モニタリング、UMIN000008696”を実施中である。より多数例、かつ経時的に、effector Tregs の存在比率、がん精巣抗原、HTLV-1関連抗原等に対する免疫応答をモニタリングし、CCR4抗体による effector Tregs 特異的除去の臨床的意義を明らかにする必要がある。また、本研究課題ではATLを対象としたが、CCR4抗体はATLだけではなく固形腫瘍に対しても有効な可能性があり、治験(NCT01929486)が進行中である。Effector Tregs 特異的除去を作用機序とする、がん免疫賦活薬としてのCCR4抗体の、今後の展開が期待される。

E．結論

HTLV-1 無症候性キャリア、ATL 患者ともに、がん・精巣抗原特異的免疫応答を獲得しているケースを認め、この免疫応答を増強させることにより ATL の発症予防法、あるいは ATL に対する新規治療法につながる可能性が示唆された。2012 年に世界に先駆け日本で、再発又は難治性の ATL に承認された CCR4 抗体は Tregs のうち effector Tregs を特異的に除去する。この機序により HTLV-1 関連抗原のみならず、NY-ESO-1 など、がん精巣抗原に対する免疫応答が増強される可能性がある。すなわち、がん精巣抗原を標的とする免疫療法と CCR4 抗体療法の併用療法は ATL の発症予防法、あるいは ATL に対する新規治療法として有望であることが示唆された。

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G．知的所有権の取得状況

1．特許取得

該当なし

2．実用新案登録

該当なし

3.その他

該当なし

別紙４

研究成果の刊行に関する一覧表レイアウト

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