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# RECIRKULACIJA NAIVNIH T LIMFOCITA

**Sažetak:** Nakon razvoja u timusu, naivni T limfociti dospijevaju u cirkulaciju i kontinuirano recirkulišu između krvi i perifernih limfoidnih organa u cilju aktivacije i transformacije u efektorske ćelije. Kretanje naivnih T limfocita predstavlja uređen slijed kontrolisan ekspresijom specifičnih proteina (selektina, integrina i hemokina), koji uključuje regrutovanje cirkulišućih limfocita na luminalnoj površini krvnog suda, transendotelnu tranziciju, te migraciju unutarekstravaskularnog odeljka perifernih limfoidnih organa.

Pitanje kretanja naivnih T limfocita u i iz nelimfoidnih organa u fiziološkim uslovima nije u potpunosti razriješeno. Postoji mišljenje da naivni T limfociti u fiziološkim uslovima rutinski pristupaju gotovo svim nelimfoidnim organima u svrhu imunološkog nadzora i/ili indukcije tolerancije.

Nelimfoidni organi opterećeni hroničnom upalom i tumorskim procesom mogu posjedovati značajan broj naivnih T limfocita. Organizovano limfoidno tkivo uzročno doprinosi perzistenciji određenih autoimunih bolesti. Regrutacija u tumorskom tkivu i naknadni antitumorski imunološki odgovor korespondiraju sa pozitivnom prognozom.

**Ključne riječi**: naivni T limfociti, primarni imunološki odgovor

## *Naivni T limfociti*

T limfociti su limfociti koji u imunološkim reakcijama učestvuju neposrednim ubijanjem ćelija koje izražavaju za njih specifičan antigen, te proizvodnjom i lučenjem limfokina pomoću kojih upravljaju djelovanjem drugih leukocita.[1] Eksprimuju klonalno raspoređene, polimorfne, T ćelijske receptore (engl. T Cell Receptor, TCR) koji detektuju peptidne fragmente proteinskih antigena predstavljenih u sklopu molekula glavnog kompleksa histokompatibilnosti (engl. major histocompatibility complex, MHC).[2]

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Naivni T limfociti su nezreli T limfociti koji su uspješno završili pozitivnu i negativnu selekciju u timusu ali nisu ostvarili kontakt sa antigenom.[3] Posjeduju malu citoplazmu, spor metabolizam i nisu u mogućnosti proizvesti proinflamatorne citokine.[4] Eksprimuju L selektin (CD62L), hemokinski receptor 7 (engl. C-C Motif Chemokine Receptor 7, CCR7), CD45RA (TEMRA), CD127 i CD132, ali ne i markere prethodne aktivacije, uključujući humani histokompatibilni leukocitni antigen klase II (engl. Human Histocompatibility Leucocyte Antigens class II, HLA-DR), CD25, CD44, CD69, CD45RO[3-5]).

Naivni T limfociti predstavljaju heterogenu populaciju.[5]. Funkcija timusa, starost i ukupan broj T ćelija uslovljavaju značajne razlike u fenotipu, dinamici, lokaciji, funkciji i statusu diferencijacije.[5] U prvim godinama života najveći dio naivnih T limfociti se proizvodi u timusu.[3,4,6] Insuficijentna timopoeza u odrasloj životnoj dobi se kompenzuje perifernom proliferacijom T limfocita.[3,4,6] Homeostatsko preživljavanje omogućavaju molekuli MHC-II klase, interleukin 4 (engl. Interleukin-4, IL-4) i IL-7.[4,6] Očekivani životni vijek naivnih T limfocita iznosi od 6 do 10 godina.[4]

Aktivacija naivnih T limfocita kao odgovor na antigen i njihova kasnija proliferacija i diferencijacija predstavlja primarni imunološki odgovor.[7-9] Istovremeno s obezbjeđivanjem efektorskih T ćelija, primarni imunološki odgovor stvara imunološku memoriju, koja pruža zaštitu od naknadnog izlaganja istog patogena.[1]

## *Recirkulacija naivnih T limfocita u perifernim limfoidnim organima*

Nakon razvoja u timusu, naivni T limfociti dospijevaju u cirkulaciju i kontinuirano recirkulišu između krvi i perifernih limfoidnih organa (engl. Peripheral Lymphoid Organs, PNO) u cilju aktivacije antigenima i transformacije u efektorske ćelije.[9] Kretanje limfocita predstavlja uređen slijed koji počinje regrutovanjem cirkulišućih limfocita na luminalnoj površini krvnog suda (uključujući interakciju limfocita sa endotelom, njihovo kotrljanje i na kraju čvrstu adheziju za unutrašnjost krvnog suda).[10,11] Slijedi intravaskularna migracija luminalno adheriranih limfocita (translokaciju sa početnog mesta vezivanja na odgovarajuće izlazno mjesto), transendotelna tranzicija, te migracija unutar ekstravaskularnog kompartmenta u parenhimu.[10,11] Regrutovanjem naivnih T limfocita se predominantno odvija u postkapilarnim venulama (engl. High Endothelial Venules, HEV) limfnih čvorova (engl. Lymph nodes, LN).[12] HEV posjeduju karakterističnu morfologiju, zadebljan apikalni glikokaliks i bazalnu laminu sa fibroblastnim retikularnim ćelijama (engl. Fibroblastic Reticular Cell, FRC).[12] Osim toga, endotel HEV-a eksprimuje molekule iz porodice adresina perifernih limfnih čvorova (engl. Peripheral lymph Node Addressin, PNAd) koji predstavljaju ligande L-selektinu (Slika 1).[12,13]

Porodica PNAd uključuje molekul ćelijske adhezije-1 ovisan o glikolizaciji (engl. Glycosylation-dependent cell adhesion molecule-1, GlyCAM-1)[4,] sijalizirani glikoprotein od 200 kilodaltona (engl. sialylated glycoprotein of 200 kDa, sgp200),

podokaliksin, endomucin i nepmucin.[13] PNAd ligandi prolaze sijalilaciju, glikozilaciju, fukozilaciju razgranatog O-glikana i sulfatizaciju 6 Sialyl-Lewis X tetrasaharida (Slika 2).[13]

Endotel HEV-a u crijevnim limfoidnim tkivima, mezenterijalnim limfnim čvorovima. te endotel slezene eksprimuju mukozno adresiranu ćelijsku adhezionu molekulu-1 (engl. Mucosal vascular Addressin Cell Adhesion Molecule 1, MAdCAM-1).[14-16] MAdCAM-1 je transmembranski glikoprotein tipa I iz superfamilije imunoglobulina koji posjeduje dva domena slična imunoglobulinu i domen sličan mucinu.[14-16] Domen sličan mucinu (bogat serinom i treoninom) predstavlja ligand L selektina.[14,15]

L-selektin pripada porodici selektina, strukturno sličnih molekula ćelijske adhezije, koja uključuje i E-selektin i P-selektin (CD62E i CD62P).[17] L-selektin je transmembranski glikoprotein tipa I izražen na naivnim T limfocitima.[17] Izgrađen je od N-terminalnog, kalcijum-zavisnog lektinskog domena, domena sličnom epidermalnom faktoru rasta (engl. Epidermal Growth Factor, EGF), dva kratka ponavljajuća dijela (engl. Sequence Consensus Repeat, SCR) homologna u komplementarnim regulatornim proteinima, transmembranskog domena i citoplazmatskog repa.[18,19] L-selektin posreduje u vezivanju i kotrljanju naivnih leukocita.[12] Proces vezivanja se predominantno odvija na ugljenohidratnim epitopima lektinske domene, iako u njemu mogu učestvovati i EGF i SCR domene.[20] Zahtjeva visok stepen analogije sa endotelnim glikoproteinskim HEV ligandima.[21,22] Reverzibilna i kratkotrajna veza uzrokuje lagano kotrljanje u smjeru protoka, te interakciju s imobilizovanim hemokinima.[22] Limfoidni hemokini CCL21 i CCL19 predstavljaju protein mase od 8 do 14 kilodaltona.[23] Pripadaju grupi CC hemokina koju karakterišu dva cisteinska ostatka u NH2-terminalnom motive.[23] CCL21 i CCL19 proizvode stromalne ćelije LN i slezene.[23] CCL21 je također eksprimiran u visokim HEV i limfnim sudovima.[24]

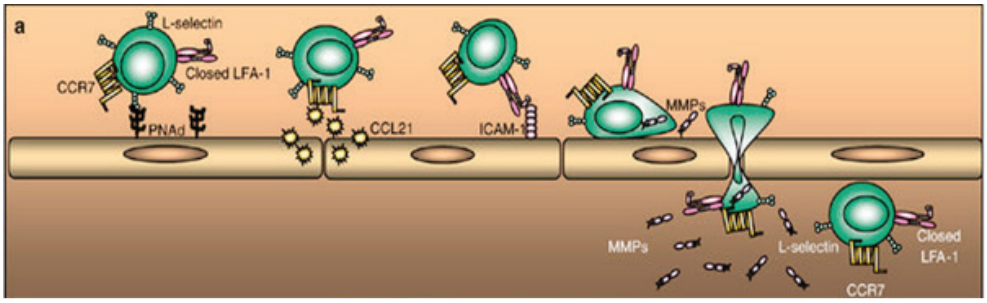
Limfoidni hemokini se vezuju za CC hemokinski receptor 7, eksprimiran na površini naivnih T ćelija i zrelih dendritskih ćelija (engl. Dendritic cells, DCs).[24] Interakcija CCL21 (i vjerovatno CCL19) HEV-a sa CCR7 omogućava konformacijsku promjenu antigena 1 povezanog sa funkcijama limfocita (engl. Lymphocyte function-associated antigen 1, LFA-1).[9]

LFA-1 integrin je glikozilirani heterodimerni molekul eksprimiran na površini naivnih T limfocita.[25] Izgrađen je od α i β podjedinice (transmembranski proteini tipa I), koje uključuju duge ekstracelularne domene, transmembransku domenu i uglavnom kratke citoplazmatske domene.[10,25] Ekstracelularna domena posjeduje ubačen (engl. Inserted, I) ligand vezujući domen, intracelularnog adhezionog molekula 1 (engl. intracellular adhesion receptor 1, ICAM-1) za snažnu adherenciju intracelularnog adhezionog molekula 1 (engl. Intercellular Adhesion Molecule 1, ICAM 1) luminalne površine HEV.[18]

U naivnim T limfocitima LFA-1 je predominantno u savijenom konformacionom obliku.[10] Aktivacijom CCR7 G-protein-vezujućeg receptora (engl. G-protein-coupled receptora, GPCR) u prisustvu hemokina latentni oblik LFA-1 prelazi u intermedijarnu

konfiguraciju (I domen umjerenog afiniteta).[10] U fiziološki perfuzovanim mikrožilama LFA-1 se brzo stabilizuje u potpuno aktivan oblik sa I domenom visokog afiniteta koji u interakciji ICAM-1 posreduje u zaustavljanju naivnih T limfocita na ICAM-1.[10]

**Slika 1. Migracija naivnih T limfocita u limfne čvorove**

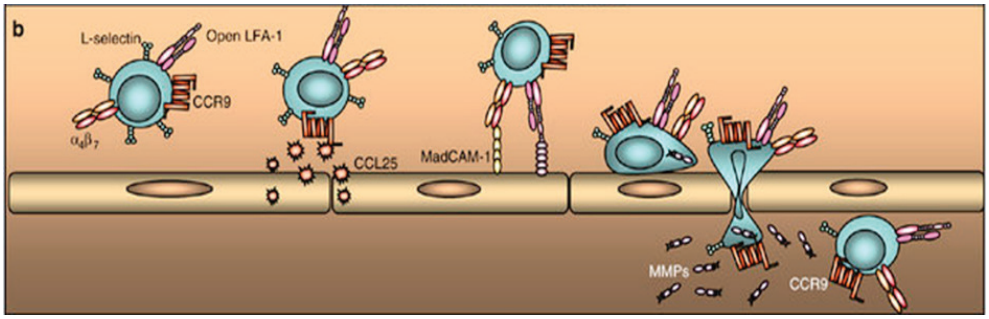



Preuzeto iz: Lewis M, Tarlton JF, Cose S. Memory versus naive T-cell migration. Immunol Cell Biol. 2008 Mar-Apr; 86(3): 226–31

Migracija naivnih T limfocita u crijevnim limfoidnim tkivima zahtjeva interakciju konstitutivno eksprimiranog integrina naivnih T limfocita, α4β7, i MAdCAM-1 HEV-a.[16] S druge strane, migracija u mezenterijalnim limfnim čvorovima podrazumjeva sekvencijalnu i sinergističku aktivnost PNAd i α4β7.[16] Interakcija PNAd/L selektin indukuje inicijalno kotrljanje i adheziju naivnih T limfocita, dok α4 β7/ MAdCAM-1 kompleks omogućava čvrstu adheziju i transmigraciju.[16]

Migraciju naivnih T limfocita u slezenu olakšava angiotenzin II (angiotenzin II/ AT1 osovina).[16] Povećana koncentracija angiotenzina II u slezeni indukuje llimfocitnu ekspresiju L-selektina i CCR9, te proizvodnju CCL19 i CCL25 u slezini.[16]

**Slika 2. Migracija naivnih T limfocita u crijevnim limfoidnim tkivima**




Preuzeto iz: Lewis M, Tarlton JF, Cose S. Memory versus naive T-cell migration. Immunol Cell Biol. 2008 Mar-Apr; 86(3): 226–31

Nakon čvrstog vezanja naivnih T limfocita, počinje provlačenje između intercelularnih spojeva endotelnih stanica.[9] Hemoatraktanti limfocita (CCL19, CCL21 i CXCL12), eksprimirani na površini FRC, potiču HEV migraciju i zadržavanje naivnih T limfocita u dubokom parakorteksu LN kroz njihovu ligaciju za CCR7 i CXC hemokinski receptor 4 (engl. CXC Motif Chemokine Receptor 4, CXCR4).[26-28] Po izlasku iz HEV-a, mreža FRC omogućava putanju za migriranje unutar parakorteksa LN prema gradijentima hemokina.[26] Osim toga, FRC produkuju IL-7 koji potiče preživljavanje i homeostazu naivnih T ćelija u dubokom parakorteksu.[28] DC, profesionalni antigen prezentujuće ćelije (engl. Antigen-presenting cell, APC), poput naivnih T limfocita, koristi sistem provodnika FRC, što povećava potencijalne interakcije (procjenjena interakcija naivnih T ćelija sa DC iznosi 500/h).[27,29] Migracija DC je uslovljena gradijentom hemokina, te interakcijom receptora sličnom lektinu C tipa 2 (engl. C-type lectin-like receptor 2, CLEC-2) DC sa podoplaninom, takođe poznatim kao glikoprotein 38 (engl. glycoprotein 38, gp38).[29] Interakcija CLEC-2-gp38 rezultuje polimerizacijom aktina DC (širenje, protruziju i migraciju DC duž FRC), kao i inhibicijom gp38 signalizacije (opuštanje aktomiozinskog citoskeleta i posljedično širenje FRC).[23]

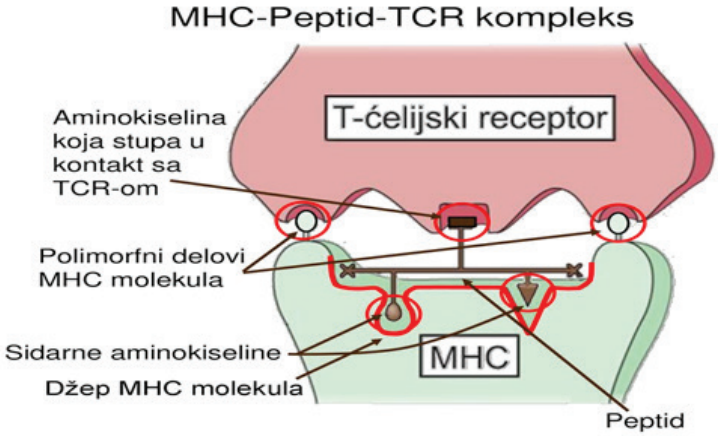
Intracelularni proteini se prikazuju u sklopu MHC molekula I klase.[28] Ekstracelularne proteine prezentuju MHC molekule II klase APC.[29]

Unutar limfnog čvora naivni T limfociti provode od 8 do 12 sati.[28] Kreću se brzinom od oko 11-14 μ u minuti.[3] S druge strane, DC putuju brzinom od oko 3-6 μ u minuti i zatim se zaustavljaju.[3] U prisustvu antigena, naivni T limfociti stupaju u interakciju sa DC, može biti prolazna (3-11 min) ili stabilna (nekoliko sati), u zavisnosti od afiniteta za antigen.[3]

Kompleks antigena i MCH molekula prepoznaju T ćelijski receptor (engl. T-cell receptor, TCR) i koreceptor.[29] Aktivacija naivnih T limfocita zahtijeva vezivanje TCR-a za peptid-MHC kompleks (signal 1), kao i interakciju kostimulatatornih molekula na interfejsu između naivnih T limfocita i DC (B7/CD28, LFA-1/ICAM-1 i ICAM2, CD2/LFA-3) (signal 2) (Slika 3).[29]

Signalni putevi aktivacije naivnih T limfocita uključuju: nuklearni faktor aktiviranih T ćelija (engl. nuclear factor of activated T cells, NFAT), RAS/RAC-mitogenom aktivirana protein kinaza (RAS/RAC mitogen-activated protein kinase, protein kinaza C, RAS/RAC MAP kinase), protein kinaza C (engl. protein kinase C, PKCθ), nuklearni faktor κB (engl. nuclear factor-κB, NF-κB), put Fosfatilinozitol-3 kinaze i kompleks tuberozne skleroze (engl. tuberous sclerosis complex, TSC).[8,30]

**Slika 3. Model prepoznavanja kompleksa peptidnog antigena i MHC molekule od strane T ćelijskog receptora**




Preuzeto iz: Abbas AK, Lichtman AH, Pillai S: Osnove imunologije. Funkcije i poremećaji imunološkog sustava. Prijevod s engleskog jezika petog izdanja knjige *Basic immunology*. Sveučilište u Splitu, Medicinski fakultet. 2016.

Prepoznavanje antigena i kostimulacija indukuju sintezu IL-2 i alfa lanca receptora za IL-2 (IL2Rα ili CD25).[8] IL-2 djeluje kao autokrini i parakrini faktor rasta koji aktivira blastogenezu ili klonsku ekspanziju.[8] Proliferaciju i preživljavanje naivnih T limfocita uključuje i interakciju Fas-Fas Ligand, faktor tumorske nekroze (engl. Tumor necrosis factor, TNF), TNF Receptor I i II, CD40-CD40 ligand, kao i perforine i interferon gama (engl. Interferon gamma, IFN-γ).[8]

Istovremeno sa proliferacijom počinje diferencija koja rezultuje stvaranjem efektorskih ($CD4^+$ pomoćnih i $CD8^+$ citotoksičnih) i memorijskih T limfocita.[8] Efektorski $CD4^+$ T limfociti (Th1, Th2, Th9, Th17, Th22) proizvode citokine i stimulišu B limfocite da proizvode antitijela.[8] Efektorski $CD8^+$ T limfociti direktno napadaju ćelije (uništavaju maligne ćelije ili ćelije inficirane virusom).[8] Memorijski T limfociti razvijaju brz odgovor pri ponovnom susretu sa antigenom.[31]

Odlazak naivnih T limfocita iz LN kontroliše fosfolipidni molekul sfingozin-1-fosfat (engl. sphingosine 1-phosphate, S1P).[9]

Unutrašnjost LN posjeduje manju koncentraciju S1P, u odnosu na krv i limfu.[9] Kada naivni T limfocit uđe u LN niske koncentracije S1P indukuje povećanu ekspresiju S1P receptora.[9] Ukoliko naivni T limfocit ne prepozna antigen on odlazi iz LN kroz eferentne limfne sudove u limfu preteći S1P gradijent.[9]

Aktivirani naivni T limfocit prolazno eksprimira CD69 koji suprimira ekspresiju S1P receptora.[9] Istim omogućava zadržavanje naivnog T limfocita u LN nekoliko dana (do završetka diferencijacije u efektorske ćelije).[9] Jednom potpuno diferentovan, efektorski T limfocit smanjuje eksprimaciju CD69, CCR7 i L-selektina i odlazi

duž gradijenta S1P kroz eferentne limfne sudove u cirkulaciju.[9] Efektorske T ćelije istovremeno eksprimiraju hemokinske receptore koji ih vode do mjesta infekcije.[9]

## *Recirkulacija naivnih T limfocita u nelimfoidnim organima*

Pitanje hominga naivnih T limfocita u i iz nelimfoidnih organa u fiziološkim uslovima nije u potpunosti razriješeno.[8,32] Postoji mišljenje da naivni T limfociti u fiziološkim uslovima rutinski pristupaju gotovo svim nelimfoidnim organima.[8,29] Prema istim, riječ je o sekundarnoj migraciji, imajući u vidu da je broj naivnih T limfocita u nelimfoidnim organima relativno mali (daleko manji od kontingenta rezidentnih ili efektorskih memorijskih T limfocita).[29,32] Ipak, imajući u vidu protok krvi kroz većinu nelimfoidnih organa i brzinu kretanja naivnih T limfocita, smatra se da će svaki naivni T limfocit vjerovatno pristupiti svim nelimfoidnim organima mnogo puta tokom svog životnog vijeka.[8]

Naivni T limfociti identifikovani su u krvi pupčanika i jetri[32]. Osim toga, izolovani su u jetri, plućima, mozgu, koži, testisima, bubrezima, pankreasu i koštanoj srži genetski modifikovanih miševa.[32]

Smatra se da značajan dio naivnih T limfocita ulazi u nelimfoidne organe kao dio normalnog migratornog puta.[32,33] Iako neki od naivnih T limfocita eksprimiraju receptore tkivno specifičnog povratka ($CD45RA^+$ T limfociti eksprimiraju integrine crijeva $\alpha4/\beta7$), najveći dio naivnih T limfocita koji migrira u nelimfoidna tkiva to čini nasumično.[8,32] U fiziološkim uslovima, migracija naivnih T limfocita kroz nelimfoidne organe odvija se po sistemu koji nije zavisan od signalizacije posredovane hemokinskim receptorima.[8,32,33] Pretpostavlja se da kotrljanje i adheziju naivnih T limfocita u odsustvu CD11a posreduju CD44 i integrin $\alpha4\beta1$ (engl. very late antigen-4, VLA-4).[8]

Naivni T limfociti nisu konstituenti nelimfoidnih organa.[8] Oni se kreću kroz iste tokom nekoliko dana, što sugeriše da naivni T limfociti pristupaju nelimfoidnim organima u svrhu imunološkog nadzora i/ili indukcije tolerancije.[8] Osim toga, smatra se da aktivacija naivnih T limfocita može otpočeti izvan sekundarnih limfoidnih organa.[8]Postoji mišljenje da najveći dio naivnih T limfocita u nelimfoidnim organima migrira u cilju indukovanja tolerancije.[8] U prilog istom govori posredovanje periferne tolerancije na $CD4^+$ T limfocite ekspresijom liganda 1 programirane smrti parenhimskih ćelija pankreasa.[8]

Antigen specifična retencija i aktivacija naivnih $CD4^+$ T limfocita u jetri omogućena je populacijom rezidentnih fagocitnih ćelija, odnosno Kupferovim ćelijama (engl. Kupffer cells, KC).[33] KC imaju sposobnost hvatanja, razgradnje i prezentovanja (u sklopu MCH molekula II klase) antigena eksprimiranog u hepatocitima.[33]

Nelimfoidni organi opterećeni hroničnom upalom i tumorskim procesom mogu posjedovati značajan broj naivnih T limfocita.[34,35]

Hroničnu upalu karakteriše HEV koje eksprimiraju PNAd $^{+}$ i/ili MAdCAM-1, često u kontekstu tercijarnih limfoidnih organa (engl. Tertiary lymphoid organs, TLOs).[35,36] Osim toga, PNAd + krvni sudovi su identifikovani u humanim tumorima (peritoneum, pluća, potkožno tkivo).[36] Ekspresija PNAd na HEV u LN/TLO je predominantno kontrolisana signalima receptora za limfotoksin-beta (engl. Lymphotoxin β Receptor, LTβR).[35] U tumorskom tkivu ekspresija je indukovana sekrecijom IFNγ.[36]

PNAd omogućava priliv naivnih T limfocita u relativno kasnoj fazi, odnosno nakon formiranja HEV-a.[35] U inicijalnom stadijumu kronične upale/tumorskog procesa naivne T ćelije pristupaju periferiji putem krvnih sudova koji nemaju HEV-specifične vaskularnih adresina.[35,36] Venule u zahvaćenim nelimfoidnim organima eksprimuju L selektinske ligande, različite od PNAd i MAdCAM.[35] Osim toga, CXC hemokinski ligand 12/faktor 1α izveden iz stromalnih stranica (engl. C-X-C Motif Chemokine Ligand 12/stromal cell-derived factor1α) konstitutivno prisutan u endotelnim stanicama nelimfoidnih organa, povećava svoju eksprimaciju nakon upale.[34]Ekspresija CCL21 u krvnim sudovima omogućava priliv naivnih T limfocita i moguće drugih CCR7$^{+}$ limfocita.[35] Aktivacija naivnih CD4$^{+}$ T limfocita rezultuje eksprimacijom limfotoksina α1β2 koji indukuje formiranje organizovanog limfoidnog tkiva.[34]

*De novo* formiranje organizovanog limfoidnog tkiva je prisutno u reumatoidnom artritisu, multiploj sklerozi, Hašimotovom tireoiditisu, dijabetes melitusu, hroničnim upalnim bolestima crijeva, kao i nekim zaraznim bolestima.[34] Organizovano limfoidno tkivo uzročno doprinosi perzistenciji autoimunih bolesti.[34] S druge strane, gustina HEV ili prisustvo TLO u humanim tumorima korespondira sa pozitivnom prognozom.[35] Samim tim, indukcija razvoja HEV-a u tumorima može biti vrijedna terapijska intervencija.[35]

## *Rezime*

U fiziološkim uslovima, naivne T ćelije cirkulišu kroz sekundarne limfoidne organe povećavajući mogućnost susreta sa antigenom.[9] Cirkulacija naivnih T limfocita predstavlja uređen slijed kontrolisan ekspresijom specifičnih proteina (selektina, integrina i hemokina), koji uključuje regrutovanje cirkulišućih limfocita na luminalnoj površini krvnog suda, transendotelnu tranziciju, te migraciju unutar ekstravaskularnog odeljka perifernih limfoidnih organa.[10]

Prepoznavanje antigena u sekundarnim limfoidnim organima indukuje aktivaciju i diferencijaciju naivnih T limfocita u efektorske i memorijske T limfocite.[3] Aktivacija i diferencijacija naivnih T limfocita zahtijeva interakciju T ćelijskog receptora sa peptidnim antigenom, signalizaciju putem kostimulatornih molekula i prisustvo citokina.[3] Jednom potpuno diferentovani, efektorski T limfociti migriraju na mjesto infekcije gdje obavljaju svoju efektorsku ulogu.[9] Dugoživeći memorijski T limfociti razvijaju brz odgovor pri ponovnom susretu sa antigenom.[9] Smatra se da naivni T lim-

fociti u fiziološkim uslovima pristupaju nelimfoidnim organima u svrhu imunološkog nadzora i/ili indukcije tolerancije.[8] U autoimunim bolestima autoantigeni mogu biti predstavljeni naivnim T limfocitima na mjestu upale, što uzročno doprinosi perzistenciji bolesti.[34] S druge strane, prisustvo naivnih T limfocita u humanim tumorima korespondira sa pozitivnom prognozom.[35]

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# RECIRCULATION OF NAIVE T LYMPHOCYTES

**Abstract:** After development in the thymus, naive T lymphocytes come into circulation and continuously recirculate between the blood and peripheral lymphoid organs for activation and transformation into effector cells. The movement of naive T lymphocytes represents an ordered sequence controlled by the expression of specific of specific proteins (selectin, integrin and chemokine) that includes the recruitment of circulating lymphocytes on the luminal surface of the blood vessel, transendothelial transition and migration within the extravascular compartment of peripheral lymphoid organs.

The question of the movement of naive T lymphocytes in and out of non-lymphoid organs in physiological conditions has not been fully resolved. There is an opinion that naive T lymphocytes under physiological conditions routinely access almost all non-lymphoid organs for the purpose of immunosurveillance and/or tolerance induction.

Non-lymphoid organs burdened by chronic inflammation and tumor processes may possess a significant number of naive T lymphocytes. Organized lymphoid tissue causally contributes to the persistence of certain autoimmune diseases. Recruitment in tumor tissue and subsequent antitumor immune response correspond with a positive prognosis.

**Keywords**: naive T lymphocytes, primary immune respon**se**

## *Naive T lymphocytes*

T lymphocytes are lymphocytes that participate in immune reactions by directly killing cells that express a specific antigen for them, and by producing and secreting lymphokines by which they control the activity of other leukocytes[1]. They express clonally distributed, polymorphic T cell receptors (TCR) that detect peptide fragments of protein antigens presented as part of major histocompatibility complex (MHC) molecules[2].

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Naive T lymphocytes are immature T lymphocytes that have successfully completed positive and negative selection in the thymus but have not made contact with the antigen[3]. They have small cytoplasm, slow metabolism and are unable to produce pro-inflammatory cytokines[4]. They express L selectin (CD62L), chemokine receptor 7 (CCR7), CD45RA (TEMRA), CD127 and CD132, but not markers of previous activation, including human histocompatibility leukocyte antigen class II (HLA-DR), CD25, CD44, CD69, CD45RO[3-5].

Naïve T lymphocytes represent a heterogeneous population[5]. Thymus function, age and total number of T cells cause significant differences in phenotype, dynamics, location, function and differentiation status[5]. In the first years of life, most of the naive T lymphocytes are produced in the thymus[3,4,6]. Insufficient thymopoiesis in adulthood is compensated by peripheral proliferation of T lymphocytes[3,4,6]. Homeostatic survival is enabled by MHC-II class molecules, interleukin 4 (English Interleukin-4, IL-4) and IL-74[6]. The life expectancy of naive T lymphocytes is from 6 to 10 years[4].

Activation of naïve T lymphocytes in response to antigen and their subsequent proliferation and differentiation represents the primary immune response[7-9]. Simultaneously with the provision of effector T cells, the primary immune response creates immune memory, which provides protection against subsequent exposure to the same pathogen[1].

## *Recirculation of naive T lymphocytes in peripheral lymphoid organs*

After development in the thymus, naive T lymphocytes reach the circulation and continuously recirculate between the blood and peripheral lymphoid organs (PNO) in order to be activated by antigens and transformed into effector cells[9]. The movement of lymphocytes represents an ordered sequence that begins with the recruitment of circulating lymphocytes on the luminal surface of the blood vessel (including the interaction of lymphocytes with the endothelium, their rolling and finally firm adhesion to the inside of the blood vessel)[10,11]. This is followed by intravascular migration of luminally adhered lymphocytes (translocation from the initial binding site to the corresponding exit site), transendothelial transition and migration within the extravascular compartment in the parenchyma[10,11]. The recruitment of naïve T lymphocytes takes place predominantly in post-capillary venules (HEV) of lymph nodes (LN)[12]. HEVs have a characteristic morphology, a thickened apical glycocalyx and a basal lamina with fibroblastic reticular cells (FRC)[12]. In addition, the endothelium of HEV expresses molecules from the peripheral lymph node addressin family (PNAd), which are ligands for L-selectin (Figure 1)[12,13].

The PNAd family includes Glycosylation-dependent cell adhesion molecule-1 (GlyCAM-1) 4, sialylated glycoprotein of 200 kDa (sgp200), podocalyxin, endomucin and nepmucin 13. PNAd ligands undergo sialylation, glycosylation, fucosylation of branched O-glycan and sulfation of 6 Sialyl-Lewis X tetrasaccharides (Figure 2)[13].

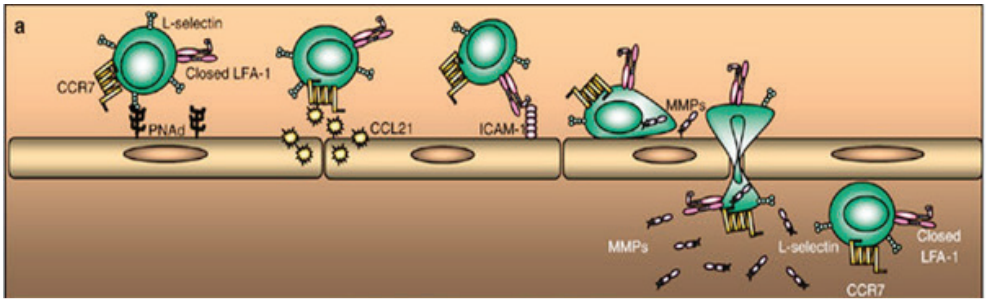
HEV endothelium in intestinal lymphoid tissues, mesenteric lymph nodes and spleen endothelium express Mucosal vascular Addressin Cell Adhesion Molecule 1, MAdCAM-[114-16]. MAdCAM-1 is a type I transmembrane glycoprotein from the immunoglobulin superfamily that possesses two immunoglobulin-like domains and a mucin-like domain[14-16]. The mucin-like domain (rich in serine and threonine) represents the ligand of L selectin[14,15].

L-selectin belongs to the selectin family, structurally similar cell adhesion molecules, which includes both E-selectin and P-selectin (CD62E and CD62P)[17]. L-selectin is a type I transmembrane glycoprotein expressed on naïve T lymphocytes[17]. It is composed of an N-terminal, calcium-dependent lectin domain, a domain similar to Epidermal Growth Factor (EGF), two short repeating parts (SCR) homologous to complementary regulatory proteins, a transmembrane domain and cytoplasmic tail [18,19]. L-selectin mediates binding and rolling of naïve leukocytes[12]. The binding process predominantly takes place on carbohydrate epitopes of the lectin domain, although EGF and SCR domains can also participate in it[20]. It requires a high degree of analogy with endothelial glycoprotein HEV ligands[21,22]. The reversible and short-lived bond causes a gentle rolling in the flow direction and interaction with immobilized chemokines[22]. Lymphoid chemokines CCL21 and CCL19 represent a protein with a mass of 8 to 14 kilodaltons[23]. They belong to the group of CC chemokines characterized by two cysteine residues in the NH2-terminal motif[23]. CCL21 and CCL19 are produced by LN and spleen stromal cells[23]. CCL21 is also expressed in high HEV and lymphatic vessels[24]. Lymphoid chemokines bind to the CC chemokine receptor 7, expressed on the surface of naive T cells and mature dendritic cells (DCs)[24]. The interaction of CCL21 (and probably CCL19) of HEV with CCR7 enables the conformational change of lymphocyte function-associated antigen 1 (LFA-1)[9].

LFA-1 integrin is a glycosylated heterodimeric molecule expressed on the surface of naïve T lymphocytes[25]. It is composed of α and β subunits (type I transmembrane proteins), which include long extracellular domains, a transmembrane domain and mostly short cytoplasmic domains[10,25]. The extracellular domain has an inserted (eng. Inserted, I) ligand binding domain of intracellular adhesion molecule 1 (eng. intracellular adhesion receptor 1, ICAM-1) for strong adherence of the intracellular adhesion molecule 1 (eng. Intercellular Adhesion Molecule 1, ICAM 1) to the luminal surface HEV[18].

In naïve T lymphocytes, LFA-1 is predominantly in a folded conformational form[10]. Upon activation of CCR7 G-protein-coupled receptor (GPCR) in the presence of chemokines, the latent form of LFA-1 changes to an intermediate configuration (I domain of moderate affinity)[10]. In physiologically perfused microvessels, LFA-1 is rapidly stabilized into a fully active form with a high-affinity I domain that interacts with ICAM-1 to mediate arrest of naïve T lymphocytes on ICAM-1[10].

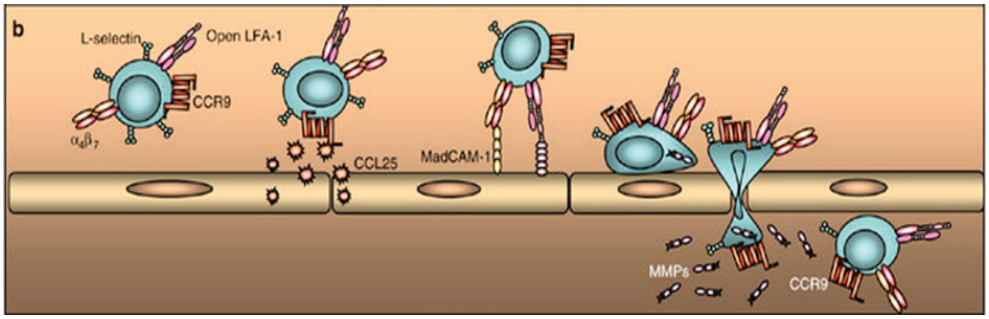
**Figure 1. Migration of naïve T lymphocytes to lymph nodes**




Taken from: Lewis M, Tarlton JF, Cose S. Memory versus naive T-cell migration. Immunol Cell Biol. 2008 Mar-Apr; 86(3): 226–31

Migration of naïve T lymphocytes in intestinal lymphoid tissues requires the interaction of the constitutively expressed integrin of naïve T lymphocytes, α4β7, and MAdCAM-1 HEV[16]. On the other hand, migration in mesenteric lymph nodes implies sequential and synergistic activity of PNAd and α4β7[16]. The PNAd/L selectin interaction induces the initial rolling and adhesion of naïve T lymphocytes, while the α4 β7/ MAdCAM-1 complex enables firm adhesion and transmigration[16]. Migration of naïve T lymphocytes in the spleen is facilitated by angiotensin II (angiotensin II/AT1 axis)[16].

**Figure 2. Migration of naïve T lymphocytes in intestinal lymphoid tissues**




Taken from: Lewis M, Tarlton JF, Cose S. Memory versus naive T-cell migration. Immunol Cell Biol. 2008 Mar-Apr; 86(3): 226–31

Increased concentration of angiotensin II in the spleen induces lymphocyte expression of L-selectin and CCR9, and production of CCL19 and CCL25 in the spleen[16].

After the tight binding of naïve T lymphocytes, they begin to move between the intercellular junctions of endothelial cells9. Lymphocyte chemoattractants (CCL19, CCL21 and CXCL12) expressed on the surface of FRC promote HEV migration and retention of naive T lymphocytes in the deep paracortex of LN through their ligation for CCR7 and CXC Chemokine Receptor 4 (CXC Motif Chemokine Receptor 4, CXCR4)[26-28]. Upon exiting the HEV, the FRC network provides a pathway to migrate within the LN paracortex according to chemokine gradients[26]. In addition, FRCs produce IL-7 which promotes the survival and homeostasis of naive T cells in the deep paracortex[28]. DC, professional antigen-presenting cells (APC), like naive T lymphocytes, use the FRC conduit system, which increases potential interactions (estimated interaction of naive T cells with DC is 500/h)[27,29]. DC migration is conditioned by the chemokine gradient and by the interaction of the C-type lectin-like receptor 2 (CLEC-2) DC with podoplanin, also known as glycoprotein 38 (gp38)[29]. CLEC-2-gp38 interaction results in polymerization of DC actin (expansion, protrusion and migration of DC along the FRC) as well as inhibition of gp38 signaling (relaxation of the actomyosin cytoskeleton and consequent expansion of the FRC)[23].

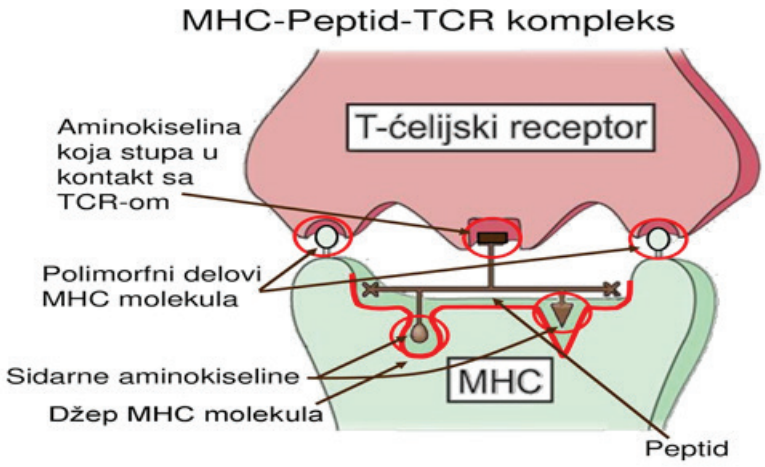
Intracellular proteins are presented as part of MHC class I molecules[28]. Extracellular proteins are presented by MHC class II molecules APC[29].

Inside the lymph node, naive T lymphocytes spend from 8 to 12 hours [28]. They move at a speed of about 11-14 μ per minute3. On the other hand DCs travel at a speed of about 3-6 μ per minute and then stop[3]. In the presence of antigen, naïve T lymphocytes interact with DC can be transient (3-11 min) or stable (several hours), depending on the affinity for the antigen[3].

The complex of antigen and MCH molecules is recognized by T cell receptor (T-cell receptor, TCR) and coreceptor[29]. Activation of naive T lymphocytes requires TCR binding to the peptide-MHC complex (signal 1) as well as the interaction of costimulatory molecules at the interface between naive T lymphocytes and DCs (B7/CD28, LFA-1/ICAM-1 and ICAM2, CD2/LFA– 3) (signal 2) (Figure 3)[29].

The signaling pathways of activation of naïve T lymphocytes include: nuclear factor of activated T cells (NFAT), RAS/RAC mitogen-activated protein kinase, protein kinase C, RAS/RAC MAP kinase), protein kinase C (eng. protein kinase C, PKCθ), nuclear factor κB (eng. nuclear factor-κB, NF-κB), Phosphatilinositol-3 kinase pathway and tuberous sclerosis complex (eng. tuberous sclerosis complex, TSC )[8,30].

**Figure 3. Model of recognition of peptide antigen complex and MHC molecule by T cell receptor**




Taken from: Abbas AK, Lichtman AH, Pillai S: Fundamentals of Immunology. Functions and disorders of the immune system. Translation from the English language of the fifth edition of the book Basic immunology. University of Split, Faculty of Medicine. 2016

Antigen recognition and co-stimulation induce the synthesis of IL-2 and the IL-2 receptor alpha chain (IL2Rα or CD25)[8]. IL-2 acts as an autocrine and paracrine growth factor that activates blastogenesis or clonal expansion[8]. Proliferation and survival of naïve T lymphocytes includes Fas-Fas Ligand interaction, tumor necrosis factor (TNF), TNF Receptor I and II, CD40-CD40 ligand, as well as perforins and interferon gamma (interferon gamma, IFN-γ)[8].

At the same time as proliferation, differentiation begins, which results in the creation of effector (CD4+ helper and CD8+ cytotoxic) and memory T lymphocytes[8]. Effector CD4+ T lymphocytes (Th1, Th2, Th9, Th17, Th22) produce cytokines and stimulate B lymphocytes to produce antibodies[8]. Effector CD8+ T lymphocytes directly attack cells (destroy malignant cells or virus-infected cells)[8]. Memory T lymphocytes develop a rapid response upon re-encounter with the antigen[31].

The departure of naïve T lymphocytes from LN is controlled by the phospholipid molecule sphingosine-1-phosphate (S1P)[9]. The interior of the LN has a lower concentration of S1P, compared to the blood and lymph[9]. When a naive T lymphocyte enters the LN, a low concentration of S1P induces an increased expression of the S1P receptor[9]. If the naive T lymphocyte does not recognize the antigen, it leaves the LN through the efferent lymphatic vessels into the lymph, threatening the S1P gradient[9].

Activated naive T lymphocyte transiently expresses CD69 which suppresses the expression of S1P receptor[9]. The same enables retention of naïve T lymphocytes in

LN for several days (until the end of differentiation into effector cells)[9]. Once fully differentiated, effector T lymphocytes reduce the expression of CD69, CCR7 and L-selectin and go along the S1P gradient through the efferent lymphatic vessels into the circulation[9]. Effector T cells simultaneously express chemokine receptors that guide them to the site of infection[9].

## *Recirculation of naive T lymphocytes in nonlymphoid organs*

The issue of homing of naïve T lymphocytes to and from non-lymphoid organs in physiological conditions has not been fully resolved[8,32]. There is an opinion that under physiological conditions naive T lymphocytes routinely access almost all non-lymphoid organs[8,29]. According to the same, it is secondary migration, bearing in mind that the number of naïve T lymphocytes in non-lymphoid organs is relatively small (far smaller than the contingent of resident or effector memory T lymphocytes)[29,32]. However, given the blood flow through most non-lymphoid organs and the speed of movement of naïve T lymphocytes, it is thought that each naive T lymphocyte is likely to access all non-lymphoid organs many times during its lifetime[8]. Naive T lymphocytes were identified in cord blood and liver[32]. In addition, they have been isolated in the liver, lungs, brain, skin, testes, kidneys, pancreas and bone marrow of genetically modified mice[32].

It is considered that a significant part of naïve T lymphocytes enters non-lymphoid organs as part of the normal migratory path[32,33]. Although some naive T lymphocytes express tissue-specific homing receptors (CD45RA+ T lymphocytes express gut integrins α4/β7), the majority of naive T lymphocytes that migrate to non-lymphoid tissues do so randomly[8,32]. In physiological conditions, the migration of naive T lymphocytes through non-lymphoid organs takes place according to a system that is not dependent on signaling mediated by chemokine receptors[8,32,33]. It is assumed that the rolling and adhesion of naïve T lymphocytes in the absence of CD11a is mediated by CD44 and integrin α4β1 (very late antigen-4, VLA-4)[8].

Naive T lymphocytes are not constituents of non-lymphoid organs[8]. They move through them over several days, suggesting that naïve T lymphocytes access non-lymphoid organs for the purpose of immunosurveillance and/or tolerance induction[8]. In addition, it is believed that the activation of naïve T lymphocytes can start outside the secondary lymphoid organs[8]. There is an opinion that the largest part of naive T lymphocytes in non-lymphoid organs migrates in order to induce tolerance[8]. This is supported by the mediation of peripheral tolerance to CD4+ T lymphocytes by the expression of ligand 1 programmed death of pancreatic parenchymal cells[8]. Antigen-specific retention and activation of naive CD4+ T lymphocytes in the liver is enabled by a population of resident phagocytic cells, i.e. Kupffer cells (KC)[33]. KCs

have the ability to capture, degrade and present (as part of class II MCH molecules) antigen expressed in hepatocytes[33].

Non-lymphoid organs burdened with chronic inflammation and tumor process may possess a significant number of naïve T lymphocytes[34,35]. Chronic inflammation is characterized by HEVs expressing PNAd + and/or MAdCAM-1, often in in the context of tertiary lymphoid organs (TLOs)[35,36]. In addition, PNAd + blood vessels have been identified in human tumors (peritoneum, lung, subcutaneous tissue)[36]. The expression of PNAd on HEV in LN/TLO is predominantly controlled by the signals of the receptor for lymphotoxin-beta (LTβR)[35]. In tumor tissue, the expression is induced by the secretion of IFNγ[36].

PNAd enables the influx of naïve T lymphocytes in a relatively late phase, i.e. after the formation of HEV[35]. In the initial stage of the chronic inflammation/ tumor process, naïve T cells access the periphery via blood vessels that do not have HEV-specific vascular addresses[35,36]. Venules in affected nonlymphoid organs express L selectin ligands, distinct from PNAd and MAdCAM[35]. In addition, CXC Chemokine Ligand 12/stromal cell-derived factor 1α constitutively present in endothelial cells of non-lymphoid organs increases its expression after inflammation[34]. The expression of CCL21 in blood vessels enables the influx of naïve T lymphocytes and possibly other CCR7+ lymphocytes[35]. Activation of naïve CD4+ T lymphocytes results in the expression of lymphotoxin α1β2, which induces the formation of organized lymphoid tissue[34].

De novo formation of organized lymphoid tissue is present in rheumatoid arthritis, multiple sclerosis, Hashimoto's thyroiditis, diabetes mellitus, chronic inflammatory bowel diseases, as well as some infectious diseases[34]. Organized lymphoid tissue causally contributes to the persistence of autoimmune diseases[34]. On the other hand, the density of HEV or the presence of TLO in human tumors corresponds with a positive prognosis[35]. Therefore, the induction of HEV development in tumors can be a valuable therapeutic intervention[35].

## *Briefly*

Under physiological conditions, naive T cells circulate through secondary lymphoid organs, increasing the possibility of antigen encounter[9]. Circulation of naïve T lymphocytes represents an ordered sequence controlled by the expression of specific proteins (selectin, integrin and chemokine) which includes the recruitment of circulating lymphocytes on the luminal surface of the blood vessel, transendothelial transition and migration within the extravascular compartment of peripheral lymphoid organs[10].

Antigen recognition in secondary lymphoid organs induces activation and differentiation of naïve T lymphocytes into effector and memory T lymphocytes[3]. The activation and differentiation of naive T lymphocytes requires the interaction of the T

cell receptor with the peptide antigen, signaling through costimulatory molecules and the presence of cytokines[3]. Once fully differentiated, effector T lymphocytes migrate to the site of infection where they perform their effector role[9]. Long-lived memory T lymphocytes develop a rapid response upon re-encounter with the antigen[9]. It is believed that naive T lymphocytes in physiological conditions approach non-lymphoid organs for the purpose of immune surveillance and/or induction of tolerance[8].

In autoimmune diseases, autoantigens can be presented by naïve T lymphocytes at the site of inflammation, which causally contributes to the persistence of the disease[34]. On the other hand, the presence of naïve T lymphocytes in human tumors corresponds to a positive prognosis[35].

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