

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO			
RAD: 12-144298- -2	FECHA: 2014-03-17 16:50:48		
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 379 FASE NACIONAL INCLUIDO CAPITULO II		
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 9		
ACT: 519 PRESENTACION			

DIRECCIÓN DE NUEVAS CREACIONES

2020
 Bogotá, D.C.,
 Abogado(a)/Señor(a)
 CARLOS REINALDO OLARTE GARCIA

Asunto: Radicación: 12-144298- -2
 Trámite: 11
 Evento: 379
 Actuación: 519
 Oficio: 3446
 Folios: 9

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I		Capítulo II	X
No. Solicitud Internacional	PC T/IB2011/050792				
Fecha de Presentación Internacional	24/02/2011				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado 12-144298-00000-0000	24/Agosto/2012
Reivindicaciones:	Radicado 12-144298-00000-0000	24/Agosto/2012
Dibujos:	Radicado 12-144298-00000-0000	24/Agosto/2012
Prioridad:	US 61 307 670	24/Febrero/2010
	US 61 438 205	12/Agosto /2010

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 17 del capítulo reivindicatorio (12-144298-00000-0000 modificado Pág. 97).

Título de la solicitud: “Anticuerpos Antagonistas Anti Receptor de IL-7 y Procedimientos”.

El problema planteado en esta solicitud consiste en la necesidad de proporcionar anticuerpos que antagonizan la actividad del receptor de interleucina-7 (IL-7R) para la prevención y/o tratamiento de diabetes tipo 2, enfermedad de injerto contra huésped (GVHD) y trastornos autoinmunes, incluyendo diabetes tipo 1, esclerosis múltiple, artritis reumatoide y lupus.

Para resolver este problema técnico la solicitud en estudio proporciona una composición farmacéutica que contiene anticuerpos de longitud completa o partes de unión a antígenos de los mismos, antagonistas de IL-7R y procedimientos para usar el antagonista de IL-7R.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07K 16/28, C12N 15/13, A61K 39/395, A61P 37/00, A61P 3/10, A61P 37/06.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 13 – 17 no es patentable de acuerdo con el Art citado.

5. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es clara porque define los anticuerpos reclamados por nombres comunes y no por su estructura, se recuerda a la solicitante que un anticuerpo debe definirse a partir de su estructura completa, esto es, de las secuencias de las regiones variables de la cadena ligera y cadena pesada.

Las reivindicaciones 4 y 5 no son claras y no tienen sustento en la descripción, ya que reclaman múltiples variantes no definidas, de las cuales la solicitante no demostró obtener todos los anticuerpos generados a partir de todas las posibles sustituciones descritas. Se sugiere a la solicitante definir el anticuerpo por su estructura, que corresponde a las cadenas ligeras y pesadas de las regiones variables y restringir las posibles variantes a una generalización razonable de lo divulgado en la descripción.

La reivindicación 4 se refiere a que el anticuerpo bloquea la fosforilación de STAT5 en un ensayo de activación de STAT5, que es un resultado a alcanzar y no define al anticuerpo, puesto que la reivindicación incluiría no sólo la solución propuesta por la solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto

La línea celular reclamada en la reivindicación 11 debe indicar su número de depósito.

La molécula de ácido nucleico reclamada en la reivindicación 12 debe ser caracterizada a partir de la secuencia nucleótida de la misma y hacer referencia a las secuencias del anticuerpo completo.

6. Unidad de invención (Art 25 D 486)

La solicitud no cumple con el requisito de unidad de invención porque se refiere a 3, grupos inventivos.

Los grupos inventivos están definidos por las siguientes características esenciales:

1° Un anticuerpo del receptor de interleucina 7 (IL-7R) aislado que se une específicamente a IL-7R y comprende una región de unión a antígeno que compite de forma cruzada, con un anticuerpo monoclonal seleccionado del grupo que consiste en P3A9, P4B3, P2E11, HAL403a, HAL403B, C1GM Y C2M3, por la unión a IL-7R

2° Un anticuerpo aislado que se une específicamente al receptor de interleucina 7 alfa (IL-7R α), en el que el anticuerpo comprende una región de determinación de complementariedad uno (CDR1) de una región variable de cadena pesada (VH) que tiene la secuencia de aminoácidos X1X2VMH, en la que X1 es D o N; X2 es S o Y (SEQ N° 50), una CDR2 de VH que tiene la secuencia de aminoácidos X1X2X3X4X5TYYADSVKG, en la que X1 es L o A; X2 es V o I; X3 es G o S; X4 es W o G; X5 es D o S; X6 es F, G o S; X7 es F, A o S (SEQ N° 51), y una CDR3 de VH que tiene la secuencia de aminoácidos X1X2X3X4X5X6X7X8 en la que X1 es Q o D; X2 es G o I; X3 es D o S; X4 es Y o G; X5 es M, V o G; X6 es G o F; X7 es N, D o M; X8 es N, Y o D (SEQ N° 52), una CDR 1 de región variable de cadena ligera (VL) que tiene la secuencia de aminoácidos TX1SSGX2IX3SSYVQ en la que X1 es R o G; X2 es S o R; X3 es D o A (SEQ N° 53), una CDR2 de VL que tiene la secuencia de aminoácidos EDX1QRPA en la que X1 es D o N (SEQ N° 53), y una CDR3 de VL que tiene la secuencia de aminoácidos X1X2X3X4X5X6X7 en la que X1 es Q o M; X2 es S o Q; X3 es D o A; X4 es F o S; X5 es H o S; X6 es H o S; X7 es V o W (SEQ N° 55), en el que el anticuerpo bloquea la fosforilación de STAT5 en un ensayo de activación de STAT5.

3° Un anticuerpo aislado que se une específicamente al receptor de interleucina 7 alfa (IL-7R α), en el que el anticuerpo comprende una región determinante de complementariedad uno (CDR1) de región variable de cadena pesada (VH) que tiene la secuencia de aminoácidos DSVMH (SEQ N°19), GFTFDDS (SEQ N° 46), o GFTFDDSVMH (SEQ N° 47), una CDR2 VH que tiene la secuencia de aminoácidos LVGWDGFFTYADSVKG (SEQ N° 23) o GWDF (SEQ N° 48), una CDR 3 de VH que tiene la secuencia de aminoácidos QGDYMGNN (SEQ ID N° 49), una CDR1 de región variable de cadena ligera (VL) que tiene la secuencia de aminoácidos TRSSGSIDSSYVQ (SEQ N° 29) una CDR2 de VL que tiene la secuencia de aminoácidos EDDQRPS (SEQ N° 31) y una CDR de VL que tiene la secuencia de aminoácidos QSYDFHHLV (SEQ ID N° 36).

Los grupos inventivos planteados no tienen unidad de invención puesto que los anticuerpos reclamados carecen de un elemento común estructural. Se le sugiere al solicitante restringir la materia a patentar en un solo concepto común inventivo especificando el avance tecnológico sobre el estado de la técnica. Esto se logra entre otras formas definiendo las características esenciales de la invención y mostrando las otras características particulares como dependientes, pero sin perjuicio de la concisión de las nuevas reivindicaciones tanto independientes como dependientes.

El solicitante también puede realizar una o más de las divisionales de acuerdo a los grupos de conceptos técnicos inventivos presentados en esta solicitud, esto claro, sin ampliar el objeto inicial de la invención.

7. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: febrero 24 de 2010 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	WO2010017468 (A1)	“Treatment of autoimmune and inflammatory disease”	11/Febrero/2010
D2	Chung Brile et al.	“Prevention of graft-versus-host disease by anti IL-7R.alpha. antibody”	15/Octubre/2007

El documento D1¹ divulga la neutralización de la actividad biológica de la IL-7 mediante anticuerpos monoclonales de unión a CD127 o IL-7 antagonistas de la fosforilación de STAT5, como métodos de tratamiento de la esclerosis múltiple y otras enfermedades autoinmunes o trastornos inflamatorios (Resumen).

El documento D2² divulga los efectos de la IL-7 en células T maduras mediante el bloqueo de un anticuerpo monoclonal anti-IL-7Rα para la eliminación de células T alorreactivas y la prevención de la enfermedad de injerto contra huésped (GVHD) (Resumen).

8. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 1 consiste en: *“Un anticuerpo del receptor de interleucina 7 (IL-7R) aislado que se une específicamente a IL-7R y comprende una región de unión a antígeno que compite de forma cruzada, con un anticuerpo monoclonal seleccionado del grupo que consiste en P3A9, P4B3, P2E11, HAL403a, HAL403B, C1GM Y C2M3, por la unión a IL-7R”* (Pág. 97).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Anticuerpo del receptor de interleucina 7 aislado que se une específicamente a IL-7R	Anticuerpo aislado que se une específicamente a IL-7R (Pág. 6, línea 11 - 15)
P3A9, P4B3, P2E11, HAL403a, HAL403B, C1GM Y C2M3	No se mencionan

El documento D1 se considera el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga anticuerpos monoclonales que se unen al receptor CD127 para inhibir la función de IL-7 (Pág. 6, línea 11 - 15).

La diferencia entre la invención, definida en la reivindicación 1 y el anticuerpo

1http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=4&adjacent=true&locale=en_EP&FT=D&date=20100211&CC=WO&NR=2010017468A1&KC=A1

2 <http://bloodjournal.hematologylibrary.org/content/110/8/2803>

antagonista CD127 divulgado de D1 consiste en que la solicitud se refiere a los anticuerpos P3A9, P4B3, P2E11, HAL403a, HAL403B, C1GM Y C2M3.

No se evidencia un efecto técnico debido a que la reivindicación en estudio no presenta una caracterización estructural, por lo tanto no se han establecido las características técnicas esenciales de los anticuerpos que permitan compararlos con el estado de la técnica.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo obtener anticuerpos alternativos que antagonizan la actividad del receptor de interleucina-7 (IL-7R) para la prevención y/o tratamiento de diabetes tipo 2, enfermedad de injerto contra huésped (GVHD) y trastornos autoinmunes, incluyendo diabetes tipo 1, esclerosis múltiple, artritis reumatoide y lupus.

Sin embargo, la obtención de anticuerpos monoclonales que se unen específicamente a IL-7R para la prevención y/o tratamiento de diabetes tipo 2, enfermedad de injerto contra huésped (GVHD) y trastornos autoinmunes ya está divulgada en el documento D1 que se refiere a anticuerpos para el tratamiento de esclerosis múltiple y otras enfermedades autoinmunes o desordenes inflamatorios que antagonizan con el mismo receptor (Resumen). La persona del oficio normalmente versada en la materia, generaría múltiples secuencias empleando técnicas convencionales y métodos de biología molecular para obtener anticuerpos que tengan la misma actividad.

En consecuencia, la persona del oficio normalmente versada en la materia, mediante prácticas de rutina diseñaría anticuerpos alternativos de acuerdo a las enseñanzas del documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud, por lo cual se considera obvia.

Conclusión:

La reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 2, 3 y 8 – 12 parcialmente, no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

La reivindicación 4 consisten en: *“Un anticuerpo aislado que se une específicamente al receptor de interleucina 7 alfa (IL-7R α), en el que el anticuerpo comprende una región de determinación de complementariedad uno (CDR1) de una región variable de cadena pesada (VH) que tiene la secuencia de aminoácidos X1X2VMH, en la que X1 es D o N; X2 es S o Y (SEQ N° 50), una CDR2 de VH que tiene la secuencia de aminoácidos X1X2X3X4X5TTYADSVKG, en la que X1 es L o A; X2 es V o I; X3 es G o S; X4 es W o G; X5 es D o S; X6 es F, G o S; X7 es F, A o S (SEQ N° 51), y una CDR3 de VH que tiene la secuencia de aminoácidos X1X2X3X4X5X6X7X8 en la que X1 es Q o D; X2 es G o I; X3 es D o S; X4 es Y o G; X5 es M, V o G; X6 es G o F; X7 es N, D o M; X8 es N, Y o D (SEQ N° 52), una CDR 1 de región variable de cadena ligera (VL)) que tiene la secuencia de aminoácidos TX1SSGX2IX3SSYVQ en la que X1 es R o G; X2 es S o R; X3 es D o A (SEQ N° 53), una CDR2 de VL que tiene la secuencia de aminoácidos EDX1QRPA en la que X1 es D o N (SEQ N° 53), y una CDR3 de VL que tiene la secuencia de aminoácidos X1X2X3X4X5X6X7 en la que X1 es Q o M; X2 es S o Q; X3 es D o A; X4 es F o S; X5 es H o S; X6 es H o S; X7 es V o W (SEQ N° 55), en el que el anticuerpo bloquea la fosforilación de STAT5 en un ensayo de activación de STAT5”.*

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Anticuerpo aislado que se une específicamente al receptor de interleucina 7 alfa	Anticuerpo aislado que se une específicamente a IL-7Rα (Pág. 2803)
Región de determinación de complementariedad uno (CDR1) de una región variable de cadena pesada (VH) que tiene la secuencia de aminoácidos X1X2VMH, en la que X1 es D o N; X2 es S o Y (SEQ N° 50), una CDR2 de VH que tiene la secuencia de aminoácidos X1X2X3X4X5TYYADSVKG, en la que X1 es L o A; X2 es V o I; X3 es G o S; X4 es W o G; X5 es D o S; X6 es F, G o s; X7 es F, A o S (SEQ N° 51), y una CDR3 de VH que tiene la secuencia de aminoácidos X1X2X3X4X5X6X7X8 en la que X1 es Q o D; X2 es G o I; X3 es D o S; X4 es Y o G; X5 es M, V o G; X6 es G o F; X7 es N, D o M; X8 es N, Y o D (SEQ N° 52), una CDR 1 de región variable de cadena ligera (VL)) que tiene la secuencia de aminoácidos TX1SSGX2IX3SSYVQ en la que X1 es R o G; X2 es S o R; X3 es D o A (SEQ N° 53), una CDR2 de VL que tiene la secuencia de aminoácidos EDX1QRPA en la que X1 es D o N (SEQ N° 53), y una CDR3 de VL que tiene la secuencia de aminoácidos X1X2X3X4X5X6X7 en la que X1 es Q o M; X2 es S o Q; X3 es D o A; X4 es F o S; X5 es H o S; X6 es H o S; X7 es V o W (SEQ N° 55)	No se mencionan

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 4 porque divulga los efectos de la IL-7 en células T maduras mediante el bloqueo de un anticuerpo monoclonal anti-IL-7Rα para la eliminación de células T alorreactivas y la prevención de la enfermedad de injerto contra huésped (Pág. 2803).

La diferencia entre la invención, definida en la reivindicación 4 y el anticuerpo antagonista anti- IL-7Rα divulgado de D2 consiste en que la solicitud se refiere a el conjunto de las 6 CDRs que podrían conformar el anticuerpo de la solicitud.

Los ejemplos no evidencian ningún efecto técnico debido a la inclusión de las múltiples variables del conjunto de las 6 CDRs que podrían conformar el anticuerpo.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo obtener anticuerpos alternativos que antagonizan la actividad del receptor de interleucina-7 α (IL-7Rα) para la prevención y/o tratamiento de diabetes tipo 2, enfermedad de injerto contra huésped (GVHD) y trastornos autoinmunes, incluyendo diabetes tipo 1, esclerosis múltiple, artritis reumatoide y lupus.

Sin embargo, la utilización de anticuerpos monoclonales que se unen específicamente a IL-7Rα para la prevención y/o tratamiento de diabetes tipo 2, enfermedad de injerto contra huésped (GVHD) y trastornos autoinmunes ya está divulgada en el documento D2 que se refiere a estos anticuerpos para la prevención de la enfermedad de injerto contra huésped (GVHD) (Resumen). La persona del oficio normalmente versada en la materia, generaría múltiples secuencias empleando técnicas convencionales y métodos de biología molecular para obtener anticuerpos que tengan la misma actividad.

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría un conjunto de 6 CDRs que podrían conformar el anticuerpo de acuerdo a las enseñanzas del documento D2 para llegar así al objeto de la reivindicación 4 de la solicitud. Por lo cual se considera obvia.

Conclusión:
La reivindicación 4 cumple el requisito de novedad (Art. 16), pero no cumple con el

requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 8 – 12 parcialmente, no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

La reivindicación 5 consisten en: “Un anticuerpo aislado que se une específicamente al receptor de interleucina 7 alfa (IL-7R α), en el que el anticuerpo comprende una región determinante de complementariedad uno (CDR1) de región variable de cadena pesada (VH) que tiene la secuencia de aminoácidos DSVMH (SEQ N°19), GFTFDDS (SEQ N° 46), o GFTFDDSVMH (SEQ N° 47), una CRD2 VH que tiene la secuencia de aminoácidos LVGWDGFFTYADSVKG (SEQ N° 23) o GWDFFF (SEQ N° 48), una CDR 3 de VH que tiene la secuencia de aminoácidos QGDYMGNN (SEQ ID N° 49), una CDR1 de región variable de cadena ligera (VL) que tiene la secuencia de aminoácidos TRSSGSIDSSYVQ (SEQ N° 29) una CRD2 de VL que tiene la secuencia de aminoácidos EDDQRPS (SEQ N° 31) y una CDR de VL que tiene la secuencia de aminoácidos QSYDFHHLV (SEQ ID N° 36)”.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Anticuerpo aislado que se une específicamente al receptor de interleucina 7 alfa	Anticuerpo aislado que se une específicamente a IL-7R α (Pág. 2803)
Región determinante de complementariedad uno (CDR1) de región variable de cadena pesada (VH) que tiene la secuencia de aminoácidos DSVMH (SEQ N°19), GFTFDDS (SEQ N° 46), o GFTFDDSVMH (SEQ N° 47), una CRD2 VH que tiene la secuencia de aminoácidos LVGWDGFFTYADSVKG (SEQ N° 23) o GWDFFF (SEQ N° 48), una CDR 3 de VH que tiene la secuencia de aminoácidos QGDYMGNN (SEQ ID N° 49), una CDR1 de región variable de cadena ligera (VL) que tiene la secuencia de aminoácidos TRSSGSIDSSYVQ (SEQ N° 29) una CRD2 de VL que tiene la secuencia de aminoácidos EDDQRPS (SEQ N° 31) y una CDR de VL que tiene la secuencia de aminoácidos QSYDFHHLV (SEQ ID N° 36)	No se mencionan

El documento D2 se considera el estado de la técnica cercano a la invención definida en la reivindicación 5 porque divulga los efectos de la IL-7 en células T maduras mediante el bloqueo de un anticuerpo monoclonal anti-IL-7R α para la eliminación de células T alorreactivas y la prevención de la enfermedad de injerto contra huésped (Pág. 2803).

La diferencia entre la invención, definida en la reivindicación 5 y el anticuerpo antagonista anti- IL-7R α divulgado de D2 consiste en que la solicitud se refiere a el conjunto de las 6 CDRs que podrían conformar el anticuerpo de la solicitud.

Los ejemplos no evidencian ningún efecto técnico debido a la inclusión de las múltiples variables del conjunto de las 6 CDRs que podrían conformar el anticuerpo

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo obtener anticuerpos alternativos que antagonizan la actividad del receptor de interleucina-7 α (IL-7R α) para la prevención y/o tratamiento de diabetes tipo 2, enfermedad de injerto contra huésped (GVHD) y trastornos autoinmunes, incluyendo diabetes tipo 1, esclerosis múltiple, artritis reumatoide y lupus.

Sin embargo, la utilización de anticuerpos monoclonales que se unen específicamente a IL-7R α para la prevención y/o tratamiento de diabetes tipo 2, enfermedad de injerto contra huésped (GVHD) y trastornos autoinmunes ya está

divulgada en el documento D2 que se refiere a estos anticuerpos para la prevención de la enfermedad de injerto contra huésped (GVHD) (Resumen). La persona del oficio normalmente versada en la materia, generaría múltiples secuencias empleando técnicas convencionales y métodos de biología molecular para obtener anticuerpos que tengan la misma actividad.

En consecuencia, la persona del oficio normalmente versada en la materia, incluiría un conjunto de 6 CDRs que podrían conformar el anticuerpo de acuerdo a las enseñanzas del documento D2 para llegar así al objeto de la reivindicación 5 de la solicitud. Por lo cual se considera obvia.

Conclusión:

La reivindicación 5 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

Puesto que las reivindicaciones dependientes 6, 7 y 8 – 12 parcialmente, no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

9. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2010017468 (A1)	1 – 12	Nivel inventivo
D2	Chung Brile <i>et al.</i>	1 – 12	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-03-25

Elaboró: Sindy Johana Brochero Ballesteros
Revisó: Sandra Carolina Crespo

blood

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Prepublished online June 26, 2007;
doi:10.1182/blood-2006-11-055673

Prevention of graft-versus-host disease by anti-IL-7R α antibody

Brile Chung, Eric P. Dudl, Dullei Min, Lora Barsky, Nancy Smiley and Kenneth I. Weinberg

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Plenary paper

Prevention of graft-versus-host disease by anti-IL-7R α antibodyBrile Chung,¹ Eric P. Dudl,² Dullei Min,¹ Lora Barsky,² Nancy Smiley,² and Kenneth I. Weinberg¹¹Division of Stem Cell Transplantation, Department of Pediatrics, Stanford University School of Medicine, Palo Alto, CA; ²Division of Research Immunology/Bone Marrow Transplantation, Childrens Hospital Los Angeles, CA

Graft-versus-host disease (GVHD) continues to be a serious complication that limits the success of allogeneic bone marrow transplantation (BMT). Using IL-7-deficient murine models, we have previously shown that IL-7 is necessary for the pathogenesis of GVHD. In the present study, we determined whether GVHD could be prevented by antibody-mediated blockade of IL-7 receptor α (IL-7R α) signaling. C57/BL6 (H2K^b) recipient mice were lethally irradiated and underwent

cotransplantation with T-cell-depleted (TCD) BM and lymph node (LN) cells from allogeneic BALB/c (H2K^d) donor mice. Following transplantation, the allogeneic BMT recipients were injected weekly with either anti-IL-7R α antibody (100 μ g per mouse per week) or PBS for 4 weeks. Anti-IL-7R α antibody treatment significantly decreased GVHD-related morbidity and mortality compared with placebo (30% to 80%). IL-7R α blockade resulted in the reduction of donor CD4⁺ or CD8⁺ T cells

in the periphery by day 30 after transplantation. Paradoxically, the inhibition of GVHD by anti-IL-7R α antibody treatment resulted in improved long-term thymic and immune function. Blockade of IL-7R by anti-IL-7R α antibody resulted in elimination of alloreactive T cells, prevention of GVHD, and improvement of donor T-cell reconstitution. (Blood. 2007; 110:2803-2810)

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Introduction

Graft-versus-host disease (GVHD) continues to be a limiting factor in the use of clinical hematopoietic stem-cell transplantation (HSCT). GVHD occurs when donor T cells recognize host antigenic disparities expressed on antigen-presenting cells (APCs), resulting in activation of alloreactive T cells and destruction of host tissues. Patients with GVHD develop a wide range of symptoms, including skin rash, diarrhea, liver disease, erythema, and weight loss, which eventually result in death.¹⁻⁷ Immunosuppressive drug treatment or mature T-cell-depleted bone marrow transplantation (TCD BMT) have been used as effective strategies to prevent GVHD.^{8,9} However, these strategies can also lead to engraftment failure, a prolonged state of immunodeficiency, and various types of opportunistic infections. Therefore, developing a therapeutic strategy to suppress GVHD without compromising the immune system will be ideal for allogeneic BMT recipients.

IL-7 and Kit ligand (KL; stem-cell factor [SCF]) are the major lymphopoietic cytokines produced in the thymus and BM compartment.¹⁰⁻¹³ IL-7 induces proliferation, differentiation, and survival of immature T lymphocytes. During normal T-cell development in the thymus, IL-7 produced by thymic epithelial cells (TECs) binds to the cognate IL-7 receptor (IL-7R). The IL-7R is composed of IL-7R α and common γ subunits and expressed on the surface of immature T-lymphoid progenitor cells. Mutations of the IL-7, IL-7R α , and γ_c genes result in defective thymopoiesis and impaired ability to produce T lymphocytes.¹⁴⁻¹⁸ Previously we and others have shown that administration of recombinant human IL-7 following histocompatible BMT in murine recipients corrects thymopoietic defects and enhances immune reconstitution, further suggesting the importance of IL-7 in the development of T lymphocytes.¹⁹ Besides its thymopoietic effects, IL-7 also promotes expansion and survival of mature naive and memory CD4⁺ and

CD8⁺ T cells. Recent studies have shown that IL-7-IL-7R interactions in concert with low-affinity interactions between T-cell receptors (TCRs) and self-peptide ligands bound to major histocompatibility complex (MHC) allow proliferation of mature T cells in the periphery.²⁰⁻²⁶ In addition, IL-7 enhances the survival of alloreactive donor T cells in allogeneic BMT recipients and plays a crucial role in the development and exacerbation of GVHD.²⁷⁻³¹

Based on the effects of IL-7 on mature T cells, we investigated whether GVHD could be prevented by a blockade of IL-7R α with an anti-IL-7R α monoclonal antibody. Similar to earlier experimental results that we obtained from the genetic model of IL-7 deficiency, we demonstrated that anti-IL-7R α antibody treatment can successfully prevent GVHD by eliminating donor mature T cells.²⁷ Paradoxically, anti-IL-7R α antibody treatment did not impair donor-derived thymopoiesis even though IL-7 is critical for the development of T cells. These results indicate that anti-IL-7R α antibody treatment may be beneficial for prevention of GVHD.

Materials and methods

Mice

Female C57BL/6J (H-2k^b, CD45.2), male B6.SJL (H-2k^b, CD45.1), male BALB/c (H-2k^d Thy 1.2), and male BALB/c (H-2k^d Thy 1.1) mice (aged 8 to 10 weeks) were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were kept in laminar flow cages with autoclaved food and acidified water. The protocol for maintaining animals before and after BMT was approved by the Childrens Hospital Los Angeles Research Institute Animal Care Committee (IACUC).

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Bone marrow transplantation procedure

Female recipient H2K^b C57BL/6J mice were given 2 separate doses of radiation (700 cGy on day -1 and 600 cGy on day 0) as previously described.²⁷ The BM from BALB/c (H2K^d Thy 1.1), BALB/c (H2K^d Thy 1.2), or B6.SJL donor mice were obtained by perfusion of the femur, and the lymph nodes (LNs) from BALB/c (H2K^d Thy 1.2) were prepared by mincing of mesenteric, axillary, and inguinal LNs. The donor BM cells were depleted for mature T lymphocytes by immunomagnetic depletion using rat antimouse Thy 1, CD4, and CD8 monoclonal antibodies (Pharmingen, San Diego, CA) and sheep antirat antibodies conjugated to beads (Dyna, Great Neck, NY). Following irradiation of recipient mice, 1×10^6 TCD BM and 4×10^6 LN cells were transplanted into recipients via tail vein injection.

Administration of anti-IL-7R α antibody

Antimurine IL-7R α antibody produced from the ST185 hybridoma clone (gift of Paul Kincade, University of Oklahoma) was purified using a HiTrap Protein G HP antibody isolation kit (GE Healthcare Bio-Sciences, Piscataway, NJ). To test whether anti-IL-7R α antibody treatment can prevent GVHD, allogeneic BMT-plus-LN recipients were subcutaneously injected weekly with either saline, nonspecific rat IgG (AbD Serotec, Raleigh, NC), or anti-IL-7R α antibody (100 μ g per mouse) from day 0 for 4 weeks. The clinical status of the BMT recipients was evaluated using a GVHD scoring system as previously described (B.C., E.D., A. Toyama, L.B., K.W., manuscript submitted, July 2007).

Cell proliferation assay

To test the anti-IL-7R α antibody's ability to block IL-7R signaling, ³H-thymidine incorporation assay was performed using the murine IL-7-dependent B-cell line, 2E8 (American Type Culture Collection [ATCC], Manassas, VA). The 2E8 cell line was incubated with either freshly purified anti-IL-7R α antibody (100 ng/mL) or nonspecific IgG for 30 minutes and treated with rhIL-7 in various different concentrations for 24 hours to measure cell proliferation.

Assessment of GVHD

GVHD severity was assessed using the previously described clinical scoring system (B.C., E.D., A. Toyama, L.B., K.W., manuscript submitted, July 2007). Each BMT recipient was scored weekly for 5 parameters (weight loss, skin integrity, fur texture, mobility, and posture) using a scale of 0 to 2, with 0 for absent or normal, 1 for mildly abnormal, and 2 for severely abnormal. The GVHD clinical index was the sum of the scores for individual criteria.

Histologic analyses

Small intestine, skin, and thymic tissues were fixed in 10% formalin, embedded in paraffin, and cut into 5 μ m-thick sections for hematoxylin and eosin (H&E) staining. Tissue sections were examined for evidence of GVHD. Immunohistochemical staining of thymic sections were done as previously described.³² The thymus from normal and BMT-recipient mice at day 30 after BMT was embedded in Tissue-Tek OCT compound (SaKura, Torrance, CA) to make frozen sections. Cryosections (5 mm) were immediately fixed with ice-cold acetone and incubated with 10% normal donkey serum (Sigma, St Louis, MO) for 10 minutes to block nonspecific binding of antibodies. Thymic sections were stained with rabbit antimouse keratin 5 (Covance Research Products, Berkeley, CA) and rat antikeratin 8 (Troma-1, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) in 1% donkey serum overnight. As a negative control, normal rabbit or rat serum (Sigma) was used. FITC-conjugated donkey antirat IgG and Cy5-conjugated donkey antirabbit IgG (Jackson Laboratory) were used as secondary antibodies. Thymic tissue sections were viewed with a Leica DM RXA-RF-8 fluorescence microscope (Leica, Bannockburg, IL) using 5 $\times$ /0.12 NA, HC Plan 10 $\times$ /0.4NA, or HC Plan 20 $\times$ /0.7 NA objective lenses for $\times$ 50, $\times$ 100, and $\times$ 200 magnification, respectively. To capture images, a SkyVision-2/VDS-1300 12-bit digital camera (Applied Spectral Imaging, Migdal HaEmek, Israel) with Easy FISH software (Applied Spectral Imaging) was used.

Immunophenotyping

BMT recipients were killed by CO₂ narcosis, and the thymus, spleen, and LN cells were removed. Single-cell suspensions of each organ were prepared, and the total cell numbers were determined. A total of 1×10^5 cells were stained with conjugated antibodies directed against Thy1.1, Thy1.2, CD4, CD8, CD44, CD45.1, CD62L, H2K^d, or isotype control monoclonal antibodies (Pharmingen). After staining, cells were washed and analyzed on the FACSCalibur (Becton Dickinson, San Diego, CA). In some experiments, donor LN cells were labeled with carboxyfluorescein diacetate succinimidyl ester (CFSE) prior to transplantation to measure the proliferation in vivo of the cells after transplantation. Donor LN cell proliferation was assessed by measuring separate peaks of decreased intensity of CFSE fluorescence upon successive cell division by fluorescence-activated cell sorter (FACS) analysis of the labeled donor CD4 or CD8 T-cell population. When cell numbers were limiting, LN cells from multiple recipients in each experimental group were pooled for analyses.

SRBC immunizations

Normal and recipient mice were immunized with sheep red blood cells (SRBCs) (Colorado Serum, Denver, CO) via intraperitoneal injection at day 44 after BMT, as previously described.¹⁹ After 2 weeks, peripheral blood was drawn for primary antibody responses against SRBCs, and the mice were boosted with SRBCs for secondary responses, which were measured 1 week later. The agglutination antibody titers in the serum were determined by serial dilution and incubation with SRBCs in 96-well V-bottom microplates (Corning, Corning, NY).

Statistical analyses

Analyses of survival rates were performed by Winstat Survival Analyses (A-Prompt, Whitehall, PA). Different immunophenotypic populations of cells following transplantation were analyzed by 2-tailed *t* test with unequal distributions. *P* values that were less than or equal to .05 were considered statistically significant.

Results

Decreased IL-7-dependent proliferation of 2E8 cell line by anti-IL-7R α antibody

Because our previous data demonstrated that proliferation and maintenance of donor-derived allogeneic mature T cells are reduced in the absence of IL-7, we investigated whether a blockade of IL-7R signaling by anti-IL-7R α antibody treatment had similar effects.²⁷ To determine whether the anti-IL-7R α antibody purified from the ST185 hybridoma supernatant binds to the murine IL-7R α , LN T cells from a BALB/c mouse were incubated in vitro with the anti-IL-7R α antibody. The purified anti-IL-7R α antibody bound to both CD4 and CD8 T cells expressing IL-7R α (Figure 1A). Next we performed a cell proliferation assay using the IL-7-dependent B-cell line, 2E8, to determine if binding of anti-IL-7R α antibody blocked IL-7-dependent proliferation. After incubating the cell line in media that contain anti-IL-7R α antibody (100 ng/mL), IL-7 was added and proliferative capacity was measured. Figure 1B shows that anti-IL-7R α antibody can effectively prevent proliferation of the 2E8 cell line. These results demonstrate that anti-IL-7R α antibody blocks IL-7R signaling and prevents IL-7-dependent cell proliferation in vitro.

Anti-IL-7R α antibody treatment prevents GVHD-related morbidity and mortality

To measure the efficacy of anti-IL-7R α antibody treatment in the prevention of GVHD, lethally irradiated (1300 cGy) B6 recipient

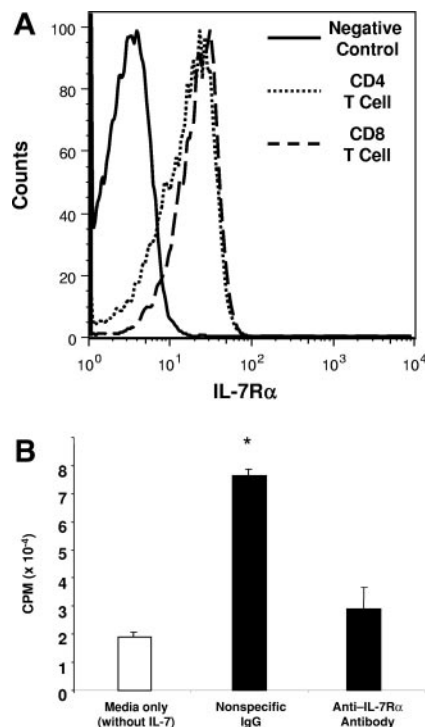


Figure 1. Blockade of IL-7R signaling by anti-IL-7R α antibody can effectively prevent proliferation of an IL-7-dependent cell line. Antimurine IL-7R α antibody purified from the ST185 hybridoma clone was tested for its ability to bind to IL-7R α and block IL-7 signaling. (A) Lymph node CD4 and CD8 T cells from a BALB/c mouse were incubated in vitro with the anti-IL-7R α antibody purified from the ST185 hybridoma supernatant. (B) To measure the effect of anti-IL-7R α antibody on cell proliferation, the IL-7-dependent murine B-cell line, 2E8, was incubated in serum-free media with either nonspecific IgG or the anti-IL-7R α antibody (100 ng/mL) for 30 minutes before IL-7 stimulation (12.5 pg/mL). For a negative control, the 2E8 cell line was incubated in media without IL-7 and IL-7R α antibody. Cell proliferation was measured by determining the rate of ^3H -thymidine incorporation into DNA for 24 hours after stimulation with or without IL-7. Asterisk indicates significant differences ($P < .05$) between PBS and anti-IL-7R α antibody-treated groups.

(H2K^b) mice underwent cotransplantation with 1×10^6 TCD BM cells and 4×10^6 LN T cells from allogeneic BALB/c mice (H2K^d). Following transplantation, either 100 μg of IL-7R α antibody or saline was intraperitoneally injected into allogeneic BMT-plus-LN recipients once a week for 4 weeks. In congenic and allogeneic BMT recipients (without LN cell administration), no mortality was observed (Figure 2A). The survival rate of allogeneic BMT-plus-LN recipients treated with saline was approximately 20% by day 150 after transplantation. In contrast, anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients remained significantly higher (70%). Although some of the anti-IL-7R α antibody-treated animals died of GVHD in the first 61 days after transplantation, no further deaths were observed after day 61.

To further measure the effects of anti-IL-7R α antibody in preventing GVHD, a GVHD scoring system was used for the first 30 days after BMT to evaluate the clinical status of mice that underwent transplantation (B.C., E.D., A. Toyama, L.B., K.W., manuscript submitted, July 2007). The recipients of either congenic or allogeneic BMT showed no signs of GVHD-related morbidity (Figure 2B). In contrast, allogeneic BMT-plus-LN recipients treated with saline displayed significant weight loss and other evidence of GVHD. Similar to the BMT-only recipients, the anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients had a significantly lower GVHD clinical score compared with saline-treated allogeneic BMT-plus-LN recipients. These results suggest that

IL-7R α antibody treatment reduces GVHD-related morbidity and mortality.

Prevention of GVHD-related tissue inflammation by anti-IL-7R α antibody

To assess the effects of anti-IL-7R α antibody on GVHD directly, GVHD target organs were analyzed on day 30 after transplantation. Sections from the skin and small intestine were stained with H&E and assessed for histologic damage. The tissue samples from allogeneic BMT-plus-LN recipients treated with saline displayed histologic evidence of GVHD. The skin samples showed lymphocytic infiltration, hyperkeratosis, and hair loss, and the intestines had villus blunting, lymphocytic infiltration, lamina propria inflammation, crypt destruction, and mucosal atrophy (Figure 3). In contrast, tissue samples from anti-IL-7R α -treated allogeneic BMT-plus-LN recipients showed no histologic evidence of GVHD and looked similar to those from normal, allogeneic, or congenic BMT recipients. These data provide evidence that anti-IL-7R α treatment can prevent GVHD-related tissue damage and inflammation.

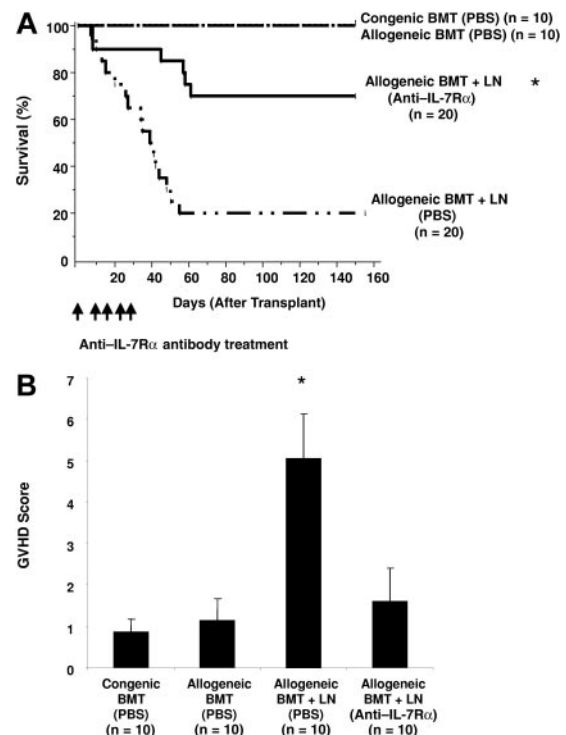


Figure 2. Anti-IL-7R α antibody treatment can successfully prevent GVHD-related mortality and morbidity following allogeneic BM transplantation. Following 1300 cGy total body irradiation (TBI), B6 recipient mice received transplants of 1×10^6 TCD BM and 4×10^6 LN cells from allogeneic BALB/c donor mice and were injected with either PBS or anti-IL-7R α antibody (100 μg per mouse once a week) subcutaneously for 4 weeks. As controls, 1×10^6 TCD BM cells from either congenic B6.SJL (CD45.1) or allogeneic BALB/c (H2K^d) donors were transplanted into lethally irradiated B6 recipients. (A) Survival of anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients is significantly greater than that of the PBS-treated allogeneic BMT-plus-LN recipients ($P < .005$). Survival over the 150 days after BMT is shown. (B) The severity of GVHD was evaluated by using a GVHD clinical grading system with scoring for 5 clinical criteria: percentage of weight loss, skin integrity, posture, mobility, and fur texture.²⁹ Clinical signs were graded on a scale of 0 to 2, where 0 was absent, 1 was moderate, and 2 was severe, and the individual signs summed. Shown are GVHD clinical index scores at day 30 following BMT. The difference between anti-IL-7R α antibody- and PBS-treated allogeneic BMT-plus-LN recipients is $P < .001$ (asterisk).

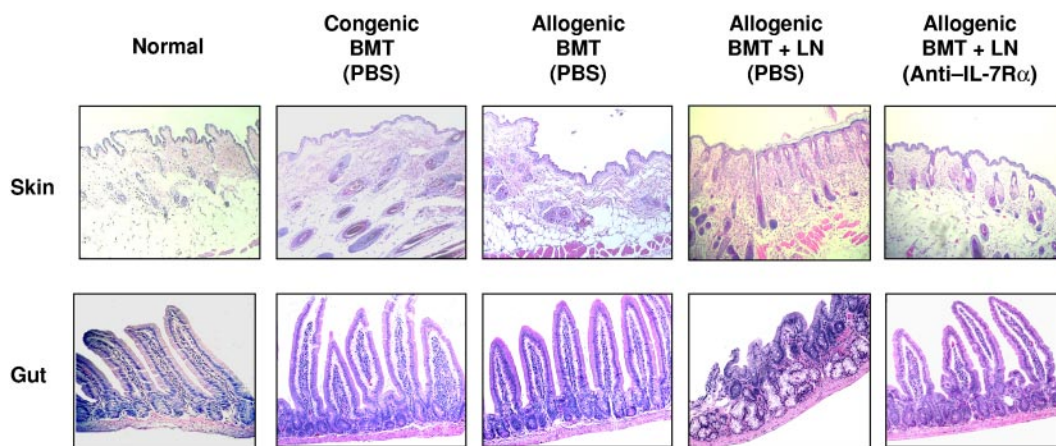


Figure 3. Histologic analysis of anti-IL-7R α antibody-treated allogeneic recipients. Skin and small intestine tissues from the recipients were analyzed at day 30 following BMT. Representative tissue samples from each group of mice were stained with H&E. The skin and small intestine from the PBS-treated allogeneic BMT-plus-LN recipients showed evidence of GVHD with lymphocytic infiltration and inflammation. The tissue sections from anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients had normal histology. See "Materials and methods, Histologic analyses" for image acquisition information.

Decreased numbers of donor T cells in the periphery of anti-IL-7R α antibody recipients

Because anti-IL-7R α antibody treatment prevented GVHD, we investigated the effects of anti-IL-7R α antibody on the survival of donor T cells in vivo. Compared with saline-treated allogeneic BMT-plus-LN recipients, the total number of donor-derived CD4 and CD8 T cells was significantly lower in the spleen and LN of anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients at days 10 and 30 after transplantation (Figure 4). To ensure that antibody treatment did not cause nonspecific IgG effects (eg, Fc receptor blockade), some allogeneic BMT-plus-LN recipients were also treated with nonspecific rat IgG antibody as a control. GVHD lethality was similar between PBS and control IgG-treated recipients (data not shown). The number of donor-derived T cells from the IgG-treated group was not decreased when compared with saline-treated recipients, and the control IgG group had significantly higher numbers of donor CD4 and CD8 T lymphocytes in the LN than anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients (Figure S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article). In addition, the kinetics of the reduction of allogeneic donor T cells in the peripheral blood of anti-IL-7R α antibody-treated recipients at days 10, 20, and 30 in these experiments showed that the reduction of donor T-cell numbers is progressive over the first 20 to 30 days after transplantation (Figure S2).

To clarify the source of the T cells observed early after transplantation, allogeneic TCD H2K^d Thy1.1⁺ BM cells underwent cotransplantation with H2K^d Thy1.2⁺ LN cells to distinguish BM-derived thymic emigrants from adoptively transferred T lymphocytes. BM-derived allogeneic T lymphocytes (Thy 1.1) at day 10 were almost undetectable in the LN of allogeneic BMT-plus-LN recipients. In contrast, most of the donor-derived T lymphocytes detected in the LN were adoptively transferred LN-derived T lymphocytes expressing Thy 1.2 (Figure S3). Therefore, early after transplantation, almost all of the analyzable T cells are derived from the cotransplanted LN cells.

Although anti-IL-7R α antibody prevents cell proliferation of the 2E8 IL-7-dependent cell line in vitro (Figure 1), the mechanism responsible for the decreased numbers of allogeneic donor T cells in vivo remained unclear. Therefore, we investigated whether inhibition of donor T-cell expansion by anti-IL-7R α antibody treatment contributes to lower numbers of CD4 and CD8 T cells in the periphery. CFSE-labeled LN cells from BALB/c donor mice were transplanted into

lethally irradiated B6 recipients treated with either anti-IL-7R α antibody or saline. At day 10, donor-derived T cells in the LNs of the recipient mice were analyzed by gating on H2K^d-positive donor T cells (Figure 5). Our data demonstrate that anti-IL-7R α antibody treatment did not completely block proliferation of donor T cells. However, we observed that the frequency of proliferating donor T cells at day 10 in the presence of anti-IL-7R α antibody was lower than that of saline-treated recipients. The reduction of donor spleen and LN T-cell numbers in anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients is

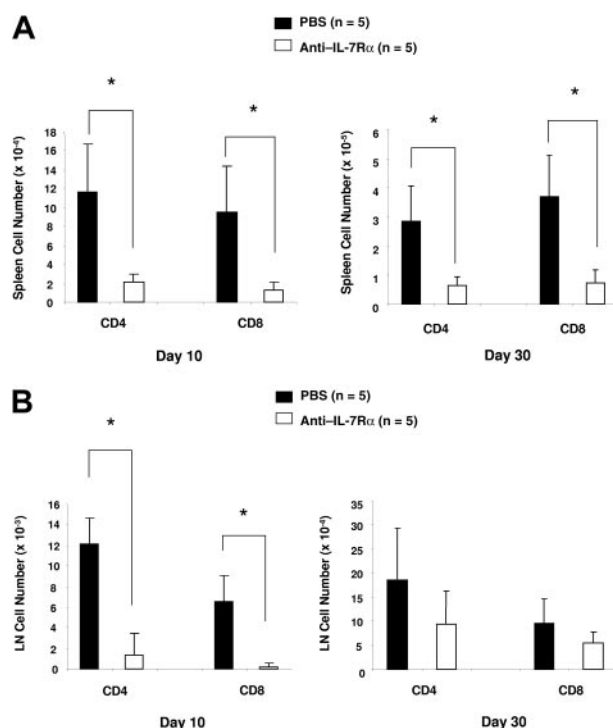


Figure 4. Administration of anti-IL-7R α antibody results in decreased numbers of donor T cells in the periphery following allogeneic BMT. Analyses of donor-derived peripheral CD4 and CD8 T-cell numbers in anti-IL-7R α antibody and PBS-treated B6 recipients were performed at day 10 and 30 following allogeneic BMT and LN-cell transplantation. Shown are numbers of donor T lymphocytes at day 10 and day 30 in spleen (A) and LN (B). Asterisk indicates significant differences between PBS- and anti-IL-7R α -treated groups (spleen and LN at day 10, $P < .02$; spleen at day 30, $P < .02$).

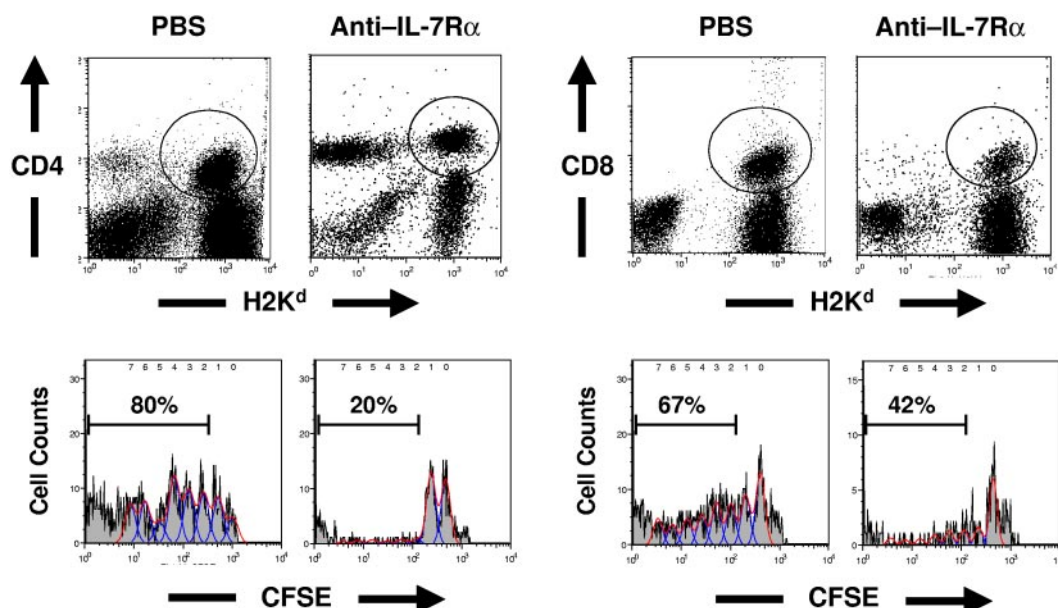


Figure 5. Administration of anti-IL-7R α antibody results in decreased proliferation of donor-derived T cells. A total of 1×10^6 TCD BM and 4×10^6 CFSE-labeled LN cells from allogeneic BALB/c donors were transplanted into lethally irradiated B6 recipients. At day 10, the frequency of proliferating CFSE-labeled allogeneic donor-derived (H2K d -positive) CD4 and CD8 T cells in LN (to the left of the indicated bar) of the anti-IL-7R α antibody-treated allogeneic BMT and LN recipients was less than that of the PBS-treated recipients.

consistent with the lower incidence of GVHD-related mortality we observed (Figure 2A).

Anti-IL-7R α antibody treatment results in improves GVHD-related thymic atrophy

TECs are targets of GVHD in both clinical and experimental allogeneic BMT.³¹⁻³³ Alloreactive donor T cells are known to damage the host thymic microenvironment. Because anti-IL-7R α antibody treatment resulted in reduction of donor-derived T cells in the periphery, we investigated whether the thymic architecture from the anti-IL-7R α antibody-treated recipients was also protected. The relative size of the thymus from saline-treated allogeneic BMT-plus-LN recipients was much smaller than that of the normal thymus and displayed no demarcation between cortical and medullary regions (Figure 6A and data not shown). In contrast, the thymic histology from the anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients did not appear to be different from that of normal, congenic, or allogeneic BMT recipients. Based on the histologic analyses, we determined whether the TECs of the anti-IL-7R α antibody-treated recipients were normal. Thymic cortical and medullary epithelial cells have been well characterized by differential expression of cytokeratins (K5 $^-$ K8 $^+$ for cortical and K5 $^+$ K8 $^-$ for medullary).³⁵ Corresponding to the H&E data, the thymus from saline-treated allogeneic BMT-plus-LN recipients displayed abnormal cortical and medullary keratin expression patterns (Figure 6B). Both K5 $^-$ K8 $^+$ cortical and K5 $^+$ K8 $^-$ medullary epithelial cells were scattered throughout the thymus, and the absence of distinctive cortico-medullary junctions was observed. In contrast, the thymus from anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients displayed normal cytokeratin expression patterns throughout the thymus resembling normal and BMT-only recipients.

The thymic tissue damage in saline-treated allogeneic BMT-plus-LN recipients suggests that donor-derived T-cell development might be also impaired. Therefore, the recipient thymus was analyzed for the relative distributions of donor thymic subpopulations at day 30 after BMT. Shown in Figure 6C are representative flow cytometric analyses of the thymus from BMT

recipients. The frequency of CD4 $^+$ CD8 $^+$ double-positive (DP) cells was greatly diminished, whereas the CD4 $^+$ CD8 $^-$ and CD4 $^-$ CD8 $^+$ single-positive (SP) cells were increased in saline-treated allogeneic BMT-plus-LN recipients when compared with those from congenic or allogeneic BMT recipients. In contrast, the relative frequencies of the donor-derived thymocyte subpopulations in anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients were similar to those seen in normal mice or BMT-only recipients. Correspondingly, the number of donor-derived thymocytes from anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients was significantly higher than saline-treated allogeneic BMT-plus-LN recipients (Figure 6D).

To investigate whether the improvement of donor-derived thymopoiesis following anti-IL-7R α antibody treatment is solely caused by the suppression of alloreactivity rather than stimulatory or beneficial effects of anti-IL-7R α antibody, we performed congenic TCD BMT (B6.SJL [H2K b CD45.1 $^+$] donors) in lethally irradiated B6 recipients (H2K b CD45.2 $^+$) and subcutaneously injected them weekly with either anti-IL-7R α antibody or nonspecific rat IgG (100 μ g per mouse) for 4 weeks. At day 30 after BMT, we observed that there is no beneficial effect in congenic hosts treated with anti-IL-7R α antibody when compared with nonspecific rat IgG-treated congenic recipients. (Figure S4). These results demonstrate that anti-IL-7R α antibody treatment not only prevents GVHD but also improves donor-derived thymopoiesis by suppression of alloreactivity.

Anti-IL-7R α antibody treatment improved development of T-cell-dependent antibody responsiveness

Because anti-IL-7R α antibody treatment preserved donor-derived thymopoiesis at day 30, we then examined functional T-cell-dependent immunity by immunizing with SRBCs the recipients that received transplants. The mice were immunized with SRBCs on day 44, and the secondary boost was given 2 weeks after primary immunization (day 58). Following immunization, we measured primary and secondary antibody responses directed

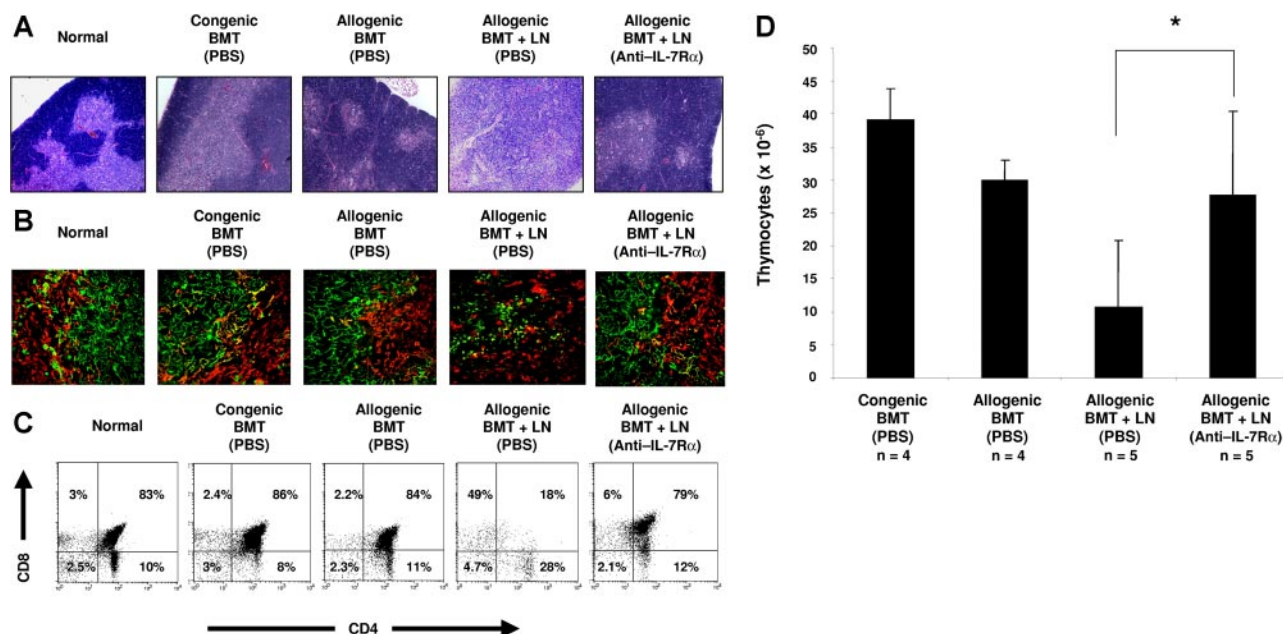


Figure 6. Anti-IL-7R α antibody treatment does not impair donor-derived thymopoiesis following allogeneic BMT. Both histologic features and donor-derived cell analysis of the thymus from recipient mice were analyzed at day 30 after BMT. Shown are representative thymuses from normal, PBS-treated congenic BM, PBS-treated allogeneic BM, PBS-treated allogeneic BMT-plus-LN, and anti-IL-7R α antibody-treated allogeneic BMT-plus-LN-cell recipient mice. (A) Similar to normal and BMT-only recipients, the thymus from anti-IL-7R α -treated allogeneic BMT and LN-cell recipients shows sharp demarcation between cortical (dense blue areas) and medullary (less dense blue areas) regions in H&E staining. Conversely, atrophic thymus from PBS-treated allogeneic BMT-plus-LN recipients displayed disorganized boundaries between cortex and medulla. (B) Thymic tissue sections from recipients receiving transplants were stained with FITC-conjugated anti-K8 (green) and PE-conjugated anti-K5 (red) antibodies to characterize the cortical (K5⁺ K8⁺) and medullary (K5⁺ K8⁻) TECs. (C) Representative FACS analyses of CD4 and CD8 expression by donor-derived thymocytes at day 30 after BMT. (D) The number of donor-derived thymocytes in BMT recipients at day 30. The difference between PBS- and anti-IL-7R α -treated allogeneic recipients is $P < .05$ (asterisk). See "Materials and methods, Histologic analyses" for image acquisition information.

against SRBCs. The saline-treated allogeneic BMT and LN transplantation group had low anti-SRBC titers after primary and secondary immunization (Figure 7). In contrast, the anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients displayed significantly higher secondary titers than the saline-treated allogeneic BMT-plus-LN recipients, and titers in the anti-IL-7R α antibody-treated recipients were comparable to those observed in the normal and BMT-only recipients.

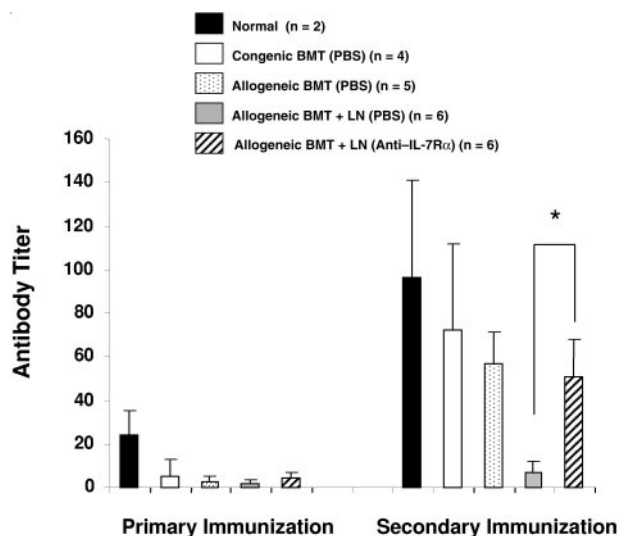


Figure 7. Anti-IL-7R α antibody treatment results in increased anti-SRBC titer following immunization. Anti-SRBC agglutinating titers were measured 1 week after the primary immunization (at day 44) and the secondary immunization (at day 58) after BMT. Asterisk indicates significant differences between PBS- and anti-IL-7R α -treated allogeneic BMT-plus-LN recipient groups ($P < .006$).

Anti-IL-7R α antibody treatment preserves donor-derived T-lymphocyte populations long-term after allogeneic BMT

Because improved thymic histology and antibody response to SRBC immunization suggest that immune reconstitution is improved in anti-IL-7R α antibody-treated recipients, we further investigated whether anti-IL-7R α antibody treatment preserves donor T lymphocytes beyond day 30 after allogeneic BMT-plus-LN transplantation. At day 250, we observed that the thymic histology and relative distributions of donor thymic subpopulations from the anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients appeared normal (Figure S5). In addition, the presence of naive donor-derived (CD62L⁺ CD44⁻) T lymphocytes in the spleen at later dates indicates the persistence of thymopoiesis. Because all the thymocytes and leukocytes in the spleen (including T cells) are donor derived, our data strongly suggest that anti-IL-7R α antibody treatment does not affect engraftment or the ability of donor hematopoietic progenitor cells to give rise to T cells.

Discussion

Because IL-7 plays a critical role in mature T-lymphocyte expansion and survival, we investigated whether a blockade of IL-7R signaling by anti-IL-7R α antibody can prevent the development of GVHD in the allogeneic setting. We observed a significantly lower incidence of GVHD-related mortality in allogeneic BMT-plus-LN recipients treated with anti-IL-7R α antibody (30%). The surviving mice remained free of GVHD for up to 8 months, even after anti-IL-7R α antibody treatment was discontinued. In contrast, 80% of saline-treated allogeneic BMT-plus-LN recipients developed GVHD and died by day 50 after transplantation. The overall GVHD clinical index score of recipients

treated with anti-IL-7R α antibody was also significantly lower than that of the saline-treated allogeneic BMT-plus-LN recipients. The anti-IL-7R α antibody treatment resulted in decreased numbers of donor-derived CD4⁺ and CD8⁺ T lymphocytes in the peripheral organs of recipients early after transplantation (before day 30). Taken together, the result of anti-IL-7R α antibody treatment is similar to that in previous B6.IL-7^{-/-} allogeneic recipients in which the absence of host IL-7 production resulted in prevention of GVHD (B.C., E.D., A. Toyama, L.B., K.W., manuscript submitted, July 2007).

Although IL-7 was initially thought to regulate T and B lymphopoiesis, IL-7 signaling is also important for the peripheral expansion and survival of mature T cells.²⁰⁻²⁶ Adoptive transfer of naive congenic T lymphocytes into sublethally irradiated IL-7^{-/-} recipients results in minimal expansion of donor T cells, which could be restored by IL-7 administration.²⁴ In addition, IL-7 mediates homeostasis of CD4 and CD8 memory T cells *in vivo*.²⁰⁻²² IL-7 signaling also promotes survival of activated allogeneic donor T cells that are prone to undergo activation-induced cell death (AICD) in early stages of GVHD.²⁷ A recent report by Zhang et al demonstrated that persistence of GVHD is caused by donor memory T cells whose homeostatic survival is enhanced by IL-7.³⁰ Because increased circulating levels of endogenous IL-7 are correlated with lymphopenia, a blockade of IL-7R signaling on alloreactive T lymphocytes in the early phases of allogeneic BMT may prevent GVHD by promoting apoptosis and suppressing expansion of host-alloreactive donor T cells.^{26,36-38} We think that the reduced numbers of donor-derived T cells in antibody-treated animals are mainly due to IL-7R α blockade function rather than via antibody-mediated cell lysis. The anti-IL-7R α antibody can effectively inhibit proliferation of the IL-7-dependent pre-B cell line, 2E8, in the presence of IL-7, as previously reported by Kincade et al.³⁹ Here, the kinetics of disappearance of peripheral donor T cells in the anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients was relatively slow compared with what would be expected if the antibody were directly mediating cell lysis. Interestingly, the observed kinetics were similar to the rate of disappearance of donor T cells in our previous allogeneic IL-7^{-/-} recipient models (B.C., E.D., A. Toyama, L.B., K.W., manuscript submitted, July 2007). Therefore, our data suggest that disappearance of donor T cells is more likely caused by blockade of IL-7R signaling than by antibody-mediated lysis.

Mice and humans with mutations in the IL-7R α or IL-7 genes have defects in thymopoiesis and a severe combined immunodeficiency (SCID) phenotype due to lack of production of mature T cells.¹⁴⁻¹⁸ Blockade of IL-7R signaling by the anti-IL-7R α antibody might have been expected to inhibit donor-derived T-cell development in the recipient thymus and delay donor immune reconstitution. Instead, we observed that anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients had improved thymic structure and cellularity compared with saline-treated allogeneic BMT-plus-LN-treated recipients. Similar to the congenic and allogeneic BMT recipients, anti-IL-7R α antibody-treated recipients displayed normal thymic structure including cortical and medullary demarcation. Donor alloreactive T lymphocytes infiltrate and damage the host thymus in experimental GVHD models.³¹⁻³³ We hypothesize that the magnitude of the thymic protective effects of the anti-IL-7R α antibody treatment exceeds any potential inhibitory effect of IL-7R blockade in the thymus. In addition, we demonstrated that the improvement of donor-derived thymopoiesis by anti-IL-7R α antibody treatment is related to suppression of GVHD rather than a thymic stimulatory effect of either control IgG or IL-7R blockade. This was shown in congenic TCD BMT recipients in which no difference in donor-derived thymopoiesis was observed following nonspecific IgG or anti-IL-7R α antibody treatment. It is also possible that the thymus-blood barrier excluded anti-IL-7R α antibody from entering the host

thymus and blocking T-cell development.⁴⁰⁻⁴² For example, Ghobrial et al demonstrated that the amount of anti-CD4 or anti-CD8 antibody required for depletion of mature T cells in the periphery was 1000-fold less than that required to cross the thymus-blood barrier to deplete thymocytes.⁴³

Following SRBC immunization, we observed that the response to a neoantigen in anti-IL-7R α antibody-treated allogeneic BMT-plus-LN recipients was significantly greater than in the saline-treated allogeneic BMT-plus-LN recipients. It is likely that the improvement of T-cell-dependent humoral immunity in the anti-IL-7R α -treated recipients was caused by donor-derived naive T lymphocytes generated from the recipient thymus. Although the low number of donor T lymphocytes in the spleen and LN of anti-IL-7R α antibody-treated recipients was observed at day 30 after allogeneic BMT-plus-LN transplantation, the subsequently improved secondary antibody responses to SRBCs are likely to be due to the increased number of peripheral donor T lymphocytes seen after cessation of anti-IL-7R treatment.

Due to the diverse TCR repertoire, naive T cells generated from the thymus have the ability to respond to a wide variety of foreign antigens more effectively than memory T cells with a limited antigenic repertoire.⁴⁴ The protection of the host thymus against donor alloreactive T cells provided by anti-IL-7R α treatment resulted in greater immunocompetence, as measured by significantly higher antibody titers directed against SRBCs after immunization. Because IL-7R α antibody-treated allogeneic BMT-plus-LN recipients showed no signs of GVHD for up to 8 months, it is likely that donor T cells developed from the thymus are not self-reactive.

Even though conventional regimens such as TCD BMT or immunosuppressive therapies can effectively prevent GVHD, delayed immune reconstitution and suppressed immune responses can often lead to fatal infections. In addition, complete depletion of donor T cells increases the risk of graft rejection by residual host T lymphocytes. Here we have demonstrated that blocking of IL-7R signaling following allogeneic BMT-plus-LN prevented GVHD while permitting immune reconstitution. The question of whether anti-IL-7R α antibody specifically targets the donor T cells responsible for causing GVHD while sparing T cells required for development of tolerance is under investigation.⁴⁵ Our data demonstrate that transient blockade of IL-7R signaling by anti-IL-7R α antibody may have therapeutic potential for prevention of GVHD.

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Authorship

Contribution: B.C. designed and performed the research, analyzed data, and wrote the paper; E.P.D. and D.M. performed the research; L.B. analyzed the data; N.S. contributed reagents; and K.I.W. designed the research and wrote the paper.

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