は，併存した癌は肺癌であり，エストロゲン標的臓器ではなかった．

再発の診断には，血中エストラジオールや腫瘍マーカーのCA125が参考になる場合があるが，本症例では異常高値は示さず有用ではなかった．一方，PET検査により，CT，MRIの骨盤内再発腫瘍部分に一致してFDGの集積を認め，再発巣の検索に有用であった．現在，顆粒膜細胞腫での再発巣の検索にPET検査をした症例の報告はほかに認めないが，今後，再発の早期発見にも有用な検査であると思われる．

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症例

ヘモグロビン2.0 g/dl未満の高度貧血を二度繰り返した子宮腺筋症の1例

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症例は49歳，主婦．2004年にヘモグロビンが1.8 g/dlの高度貧血となり，全身倦怠感を訴え受診した．輸血を行い，Gn-RHアゴニストを投与し貧血は回復した．手拳大の子宮腺筋症を認め，過多月経による貧血であった．その後も貧血を繰り返していたが，2006年に再びヘモグロビンが1.9 g/dlの高度貧血となり入院した．手術に対する恐怖心から手術を拒んでいたが，輸血を行い，Gn-RHアゴニストを投与し，貧血の回復後に子宮全摘を行った．術後の経過は良好である．

はじめに

子宮筋腫や子宮腺筋症に伴う過多月経による貧血は日常診療で頻繁に遭遇するが，ヘモグロビン(Hb)が2 g/dl未満の例はきわめて稀である[1~8]．女性の慢性の貧血に耐えうる限界は2 g/dlといわれているが[9]，そのほとんどは重症貧血に陥る前に過多月経および貧血に伴う動悸，頻脈，易疲労感などを訴え内科や婦人科を受診する．

今回われわれは，子宮腺筋症による過多月経によりHbが2.0 g/dl未満まで低下する状態を2回繰り返した例を経験したので，高度貧血を伴う症例の文献的考察を加え報告する．

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症 例

患 者：49歳，主婦．体重52.3 kg，身長162 cm

血液型：B型Rh(+)

主 訴：全身倦怠感，浮腫，歩行困難

妊娠歴：3経妊・3経産

月経歴：初経は14歳

既往歴：27歳時に骨盤腹膜炎

家族歴：特記事項なし

現病歴：2004年4月中旬に全身倦怠感，浮腫を訴え救急車で近医に入院した．血液検査で赤血球数109 × 10^4/mm^3，Hb1.8 g/dl，Ht 6.4%，MCV 58.4fl，MCH 16.7 pg，MCHC 28.6%，白血球数3,900/mm^3，血小板数20.8 × 10^4/mm^3，血清鉄6 μg/dlと高度の貧血を認めた．血液凝固，生化学検査では異常を認めなかった．入院後12単位の濃厚赤血球を2日間で輸血をした．

その後，発熱，肝脾腫を認めるため，当院に4月下旬に転院となった．手拳大の子宮腺筋症を認め，過多月経による貧血と診断し，Gn-RHアゴニスト(スプレキュア®)の点鼻を開始した．Hbが9.0 g/dlまで回復したあとは鉄剤投与で貧血は改善していき，4日後に退院した．Gn-RHアゴニスト療法後，月経の開始とともに過多月経をきたし，貧血となるため手術を勧めていたが，手術への恐怖から手術に踏み切れなかった．2003年，2004年もGn-RHアゴニストを投与し，2004年には，過多月経のためHbが4.5 g/dlまで低下し濃

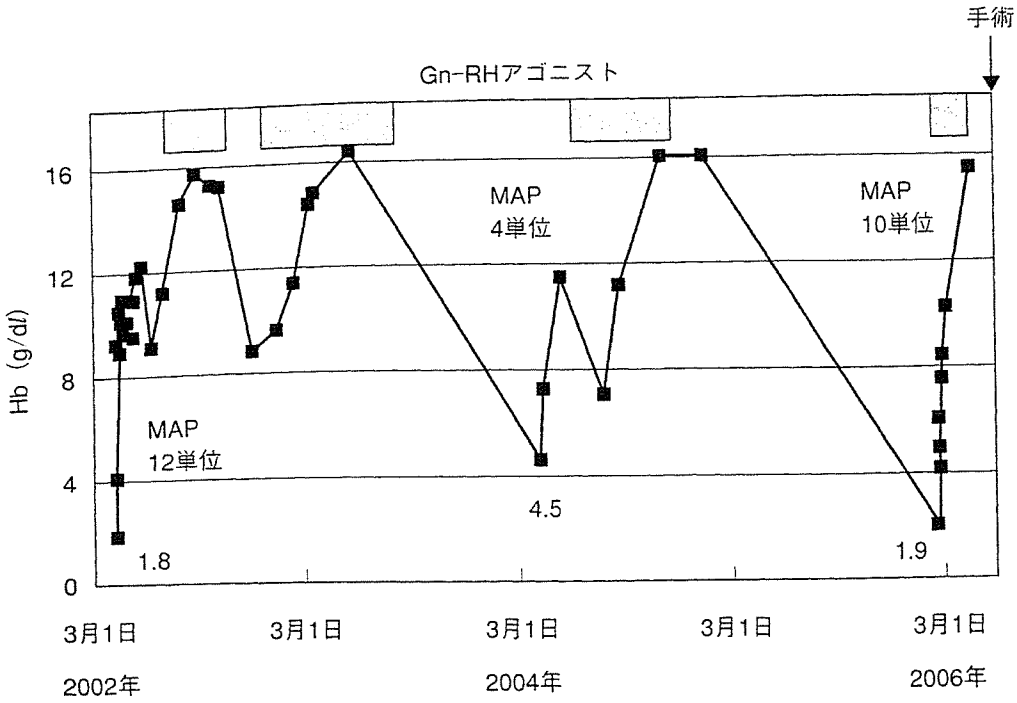

図1　症例の臨床経過

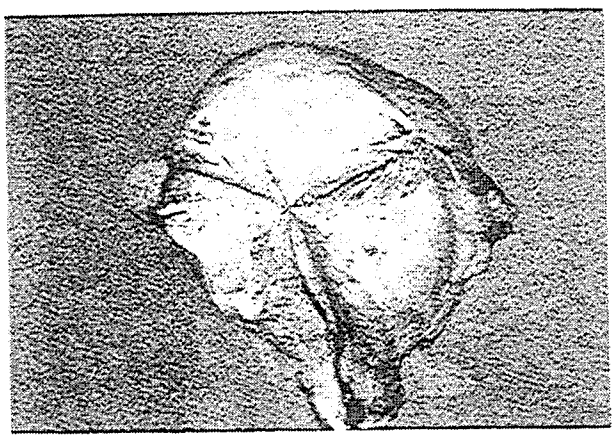

図2　摘出子宮の肉眼像

厚赤血球を4単位輸血した．その後，2005年1月以後は受診していなかった（図1）．

2006年2月下旬，全身倦怠感，浮腫，歩行困難，過多月経（約1か月）を訴え，家族に付き添われ当院内科を受診した．受診の1週間前から強い全身倦怠感を認めていた．血液検査で赤血球数 $139\times10^4/mm^3$，Hb 1.9 g/dl，Ht 8.6%，MCV 62fl，MCH 13.4 pg，MCHC 21.6%，白血球数 $2{,}400/mm^3$，血小板数 $17.9\times10^4/mm^3$，フェリチン1.2 ng/ml，血清鉄6μg/dlであり，高度の貧血を認めた．血中ビタミンB_{12}，葉酸は正常値であった．肝・腎機能，血液凝固能に異常は認めなかった．当科に緊急入院し，濃厚赤血球を4日間で10単位輸血した．入院時血圧は107/55 mmHg，脈拍89/分であった．その後，Gn-RHアゴニストの点鼻と鉄剤の服用を開始し，5月にはHbが15.4 g/dlまで回復した．

手　術：5月中旬に入院し，2日後に腹式単純子宮全摘術，両側付属器切除術を施行し，術後8日目に退院した．

切除標本・病理組織所見：摘出物は575 gであり（図2），病理診断では子宮腺筋症であった．

術後経過：術後の経過は良好で，術後7日目に退院した．

考　察

子宮筋腫や子宮腺筋症は，婦人科診療で頻度の高い疾患であり，それに伴う過多月経による貧血もしばしば認められるが，多くは重症化する前に動悸，体動時の息切れ，脱力感，易疲労感，耳鳴りなどの症状を訴え，婦人科を受診する．

女性の慢性貧血に対する生活耐用限界値は女性ではHbが2 g/dl，男性では3 g/dlとされており[9]，それ以下となることはきわめて稀である．今回の

表1　子宮筋腫・子宮腺筋症にヘモグロビン2 g/dl未満の高度貧血を伴った本邦報告例

報告者（年）	年齢（歳）	婦人科診断	Hb（g/dl）	Ht（%）	治療	症状・合併症
高瀬，他（1992）[1]	49	粘膜下筋腫，子宮内膜ポリープ	1.8	7.3	子宮全摘術	胸内苦悶，ショック状態，心拡大
小林，他（1993）[2]	44	粘膜下筋腫	1.7	—	子宮全摘術	うっ血性心不全，全身倦怠感，全身浮腫
Murao S, et al（1999）[3]	47	子宮筋腫	1.4	6	子宮全摘術	左室機能不全，心肥大，肺うっ血，全身倦怠感
山田，他（2001）[4]	52	子宮筋腫	1.9	7.0	子宮動脈塞栓術	胸水・腹水，意識障害，右片麻痺（輸血後白質脳症）
田中，他（2001）[5]	49	粘膜下筋腫	1.3	—	子宮全摘術	呼吸困難，歩行困難，心肺停止，肺水腫，意識レベルJCS 2～3
篠原，他（2002）[6]	48	子宮筋腫	1.1	4.9	子宮全摘術	食欲不振，嘔吐，心窩部痛，十二指腸潰瘍穿孔
中村，他（2004）[7]	40	粘膜下筋腫，子宮腺筋症	1.5	5.9	子宮全摘術	全身性浮腫，咳，呼吸困難，肺水腫，全身倦怠感
青江，他（2006）[8]	35	子宮筋腫	1.1	3.8	子宮全摘術	著しい心肥大，代謝性アシドーシス，意識レベルJCS 1
本症例	49	子宮腺筋症	1.8	6.4	Gn-RHアゴニスト投与	全身倦怠感，浮腫
			1.9	8.6	子宮全摘術	全身倦怠感，浮腫，歩行困難

Hb：ヘモグロビン，Ht：ヘマトクリット

症例は，Hbが1.8 g/dl，1.9 g/dlまでの高度貧血に二度陥ったのちに子宮全摘を行った．一度目の高度貧血時にも手術を強く勧められたが，手術に対する恐怖心から手術を拒否していた．

子宮筋腫や子宮腺筋症に伴い，Hbが2 g/dl未満となった本邦報告例は本症例を含め9例であった（表1）．Hbが2 g/dl未満となった症例の年齢は35歳から52歳で子宮筋腫，子宮腺筋症の好発年齢であり，子宮筋腫のなかでも粘膜下筋腫が多く9例中4例であった．治療は9例中8例に子宮全摘術が行われ，1例は動脈塞栓術が行われていた．Hbの最低値は1.1 g/dlであり，Hbが1.3 g/dlの症例では心肺停止を起こしている．そのほか，ショック状態，全身倦怠感，心不全，意識障害，浮腫，胸腹水を生じ，生命に危険が及ぶ状態となってくる．また，52歳のHbが1.9 g/dlの例では，多量の輸血後に可逆性の白質脳症により，右片麻痺となった例が報告されており[4]，多量の輸血にも注意が必要である．

しかし，Hbが2 g/dl未満でない貧血でも，合併症として，Hb 3.5 g/dlで動悸，頻脈，呼吸困難に加え輸血後肺塞栓症を生じた例[10]，Hb 3.7 g/dlで急性心筋梗塞を生じた例[11]，Hb 4.4 g/dlでせん妄を生じた例[12]，Hb 4.6 g/dlで舌痛を訴えた例[13]，Hb 5.4 g/dlで可逆性の片麻痺（左上下肢）を起こした例[14]，Hb 5.6 g/dlで若年性脳梗塞を生じた例[15]が報告されている．Hbが4～5 g/dlの貧血はたまに認められるが，このような合併症を引き起こすことがあり，貧血の予防および治療の重要性を再認識する必要がある．

また，高度の貧血の場合には，輸血が必要になる場合が多く，輸血の副作用として感染症，輸血後GVHD，アナフィラキシー反応，循環不全などが生じる可能性があるため，貧血や過多月経を有する婦人に対しては早期治療と患者の治療への啓蒙も必要である．本症例の場合，手術への恐怖心から，高度の貧血にもかかわらず全身倦怠感が耐えられなくなるまで我慢していたが，輸血のリ

スク，高度の貧血の合併症を認識させ早期に治療をする必要があったと考えられた．

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原著

子宮体癌との重複癌症例の臨床的検討

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当科で治療した子宮体癌313例中，重複癌症例は同時性癌が7例，異時性癌が23例の計30例で，子宮体癌症例全体の9.6％であった．同時性重複癌の癌種は6例が卵巣癌で，1例が子宮頸部腺癌であった．子宮体癌以前に他臓器癌を発生していた異時性重複癌は14例で，乳癌が最も多く8例，胃癌が3例と次に多く，他臓器癌との発生間隔は1年から23年であった．子宮体癌以後に他臓器癌を発生していた異時性重複癌は9例で，大腸癌が4例で最も多く，次いで乳癌が2例であった．他臓器癌との発生間隔は2年から7年であった．子宮体癌の重複癌では，特に子宮体癌以後に発生する大腸癌，乳癌に注意する必要があると思われた．

はじめに

子宮体癌は，最近増加してきている婦人科悪性腫瘍であり，子宮体癌を含む重複癌に関してはこれまでにも多くの報告がされてきた[1~10]．また，近年の高齢化に伴い重複癌の頻度も多くなると思われ，当科でも重複癌を経験する頻度が増加してきている．今回われわれは，子宮体癌を含む重複癌について臨床的に検討したので報告する．

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対象と方法

1981年1月から2004年12月までに当科で治療した子宮体癌313例（11例の癌肉腫を含む）を対象として，診療録の記載から年齢，重複癌種，組織型，発生間隔，予後などについて検討した．重複癌は，Warren & Gatesの定義[11]にしたがい，(1) 各腫瘍がそれぞれ異なった悪性像を示す，(2) 互いに離れた部位を占める，(3) 一方が他方の転移でない，という3条件を満たすものとした．また，子宮体癌と子宮頸癌，卵巣癌が独立して発生した症例も重複癌とした．子宮体癌の発症前後1年以内に重複癌を発症した例を同時性重複癌とし，1年以上の間隔がある場合を異時性重複癌症例とした[12]．

結　果

重複癌症例は同時性癌が7例，異時性癌が23例の計30例で，子宮体癌症例全体の9.6％であった．

1．子宮体癌との同時性重複癌（表1）

同時性癌は7例あり，1例は子宮頸部腺癌，6例は卵巣癌との重複癌であった．卵巣癌の6例中4例は子宮体癌と同じ類内膜癌であった．平均年齢は40.5±5.8歳であり，未妊婦が7例中3例であった．同時性重複癌の7例中6例が閉経前であった．

子宮体癌と子宮頸部腺癌との重複癌の子宮体癌の臨床進行期はIc期で，子宮頸部腺癌の臨床進行期はIIb期であった．予後は良好で，子宮頸部腺癌の再発で死亡した1例と生死不明の1例を除

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表1　子宮体癌との同時性重複癌症例

症例	年齢（歳）	経妊経産	子宮体癌		重複癌（組織型，grade）	予後
			組織型（grade）	筋層浸潤		
1	51	2妊2産	類内膜癌	1/2以上	左卵巣癌（粘液性癌）	不明
2	40	5妊3産	類内膜癌（G1）	1/2以下	子宮頸部腺癌（内頸部腺癌）	死亡（33か月）
3	42	2妊1産	類内膜癌（G2）	内膜限局	左卵巣癌（類内膜癌 G2）	生存
4	38	0妊0産	類内膜癌（G2）	1/2以下	左卵巣癌（類内膜癌 G3）	生存
5	44	0妊0産	類内膜癌（G2）	1/2以下	左卵巣癌（類内膜癌 G3）	生存
6	36	2妊2産	類内膜癌（G2）	1/2以下	左卵巣癌（類内膜癌 G2）	生存
7	33	0妊0産	類内膜癌（G1）	1/2以下	左卵巣癌（粘液性癌）	生存

表2　子宮体癌以前に他臓器癌を発生していた異時性重複癌症例

症例	年齢（歳）	他臓器癌	子宮体癌組織型（Grade）	子宮体癌治療年	他臓器癌と子宮体癌の発生間隔（年）	子宮体癌臨床進行期	子宮体癌治療後の予後
1	49	乳癌	MMT*	1990	4	IIIc	9か月死亡
2	77	胃癌	類内膜癌（G2）	1992	4	Ib	生存
3	54	S状結腸癌	類内膜癌（G2）	1990	1	Ib	生存
4	70	乳癌（術後tamoxifen服用）	類内膜癌（G1）	1996	2	Ia	生存
5	69	胃癌	類内膜癌（G2）	1999	4	Ib	生存
6	74	乳癌	類内膜癌（G2）	2000	10	IIIc	生存
7	72	胃癌	類内膜癌（G2）	2000	10	IV	60か月死亡
8	60	直腸癌	類内膜癌（G1）	2000	12	Ia	生存
9	58	乳癌	類内膜癌（G1）	2002	5	Ib	生存
10	56	卵巣Sertoli-Leydig細胞腫（術後放射線治療）	類内膜癌（G2）	2003	23	Ib	生存
11	74	乳癌	類内膜癌（G3）	2003	7	IIIc	10か月死亡
12	77	乳癌	MMT**	2003	9	Ib	生存
13	86	乳癌（第1癌），胆嚢癌（第2癌）	粘液性癌	2004	9（第1癌から）2（第2癌から）	Ib	生存
14	70	乳癌	類内膜癌（G2）	2004	10	IIIc	生存

* : mixed mesodermal tumor. heterologous type（rhabdomyosarcoma＋adenocarcinoma grade 3）

** : mixed mesodermal tumor. heterologous type（chondroosarcoma＋adenocarcinoma grade 2）

き生存している．

2．子宮体癌以前に他臓器癌を発生していた異時性重複癌（表2）

14例の子宮体癌以前に他臓器癌を発症していた異時性癌を認め，乳癌が最も多く8例で，胃癌が3例と次に多かった．子宮体癌の組織型では，類内膜癌が11例で，そのほかに粘液癌1例と2例の癌肉腫を認めた．子宮体癌治療時の平均年齢は67.5±10.5歳であった．他臓器癌との発生間隔は1年から23年であった．子宮体癌の進行期は9例がI期，4例がIII期，1例がIV期であった．三重複癌の1例は乳癌，胆嚢癌のあとに子宮体癌を発症した症例であった．予後は比較的良好で，14例中11例が生存している．

3．子宮体癌以後に他臓器癌を発生していた異時性重複癌（表3）

子宮体癌以後に発生した9例の異時性癌を認めた．癌種別では，大腸癌が4例で最も多く，次い

表3　子宮体癌以後に他臓器癌を発生していた異時性重複癌症例

症例	年齢（歳）	他臓器癌	子宮体癌 組織型（Grade）	子宮体癌 治療年	他臓器癌と子宮体癌の発生間隔（年）	子宮体癌 臨床進行期	子宮体癌治療後の予後
1	48	脳腫瘍	類内膜癌（G1）	1985	5	Ia	死亡　33か月（脳腫瘍）
2	46	横行結腸癌	類内膜癌（G1）	1989	7	Ia	生存
3	42	上行結腸癌	腺扁平上皮癌（G1）	1991	6	Ib	生存
4	45	胃癌	類内膜癌（G1）	1994	2	Ic	生存
5	63	乳癌	類内膜癌（G1）	1996	4	Ib	生存
6	65	上行結腸癌	類内膜癌（G2）	1996	6	Ic	生存
7	63	胆管癌	類内膜癌（G1）	1999	2	Ic	死亡　54か月
8	60	乳癌	類内膜癌（G3）	1999	3	Ia	生存
9	72	大腸癌	類内膜癌（G2）	1997	7	IIIa	生存

表4　子宮体癌との同時性重複癌の本邦報告例

報告者	期間（年）	体癌症例数	同時性重複癌症例数（頻度%）	重複癌種別症例数							
				卵巣癌	頸癌	大腸癌	乳癌	胃癌	腎癌	卵管癌	その他
水内ら[1]	1957～1982	129	3例（2.3%）	1	1	1	0	0	0	0	0
古久保ら[2]	1971～1992	191	7例（3.6%）	2	1	1	0	0	1	0	2*
中山ら[3]	1973～1994	486	25例（5.1%）	12	10	2	0	0	1	0	0
阿部ら[4]	1976～1992	238	16例（6.7%）	6	2	0	1	0	0	2	5**
市川ら[5]	1977～1995	147	6例（4.2%）	0	4	2	0	0	0	0	0
卜部ら[6]	1979～1992	156	6例（3.8%）	2	0	0	2	2	0	0	0
嶋本ら[7]	1984～1993	97	2例（2.1%）	0	0	0	2	0	0	0	0
後藤ら[8]	1983～1992	153	6例（3.9%）	2	3	1	0	0	0	0	0
笹川ら[9]	1982～1998	282	16例（5.7%）	8	2	1	2	2	1	0	1***
坂口ら[10]	1985～2000	261	23例（7.7%）	5	8	1	1	2	1	2	3#
自験例	1981～2004	313	7例（2.2%）	6	1	0	0	0	0	0	0
計		2,453	116（4.7%）	44	32	9	8	6	4	4	11

*：外陰癌，胸腺癌．**：卵巣癌＋卵管癌，子宮平滑筋肉腫，外陰Paget病，胆嚢癌，甲状腺癌各1例．***：肺癌．
#：耳下腺癌，尿管癌，骨肉種各1例

で乳癌が2例であった．子宮体癌の組織型では，類内膜癌が8例で，腺扁平上皮癌が1例であった．他臓器癌との発生間隔は2年から7年であった．子宮体癌治療時の平均年齢は56.0±10.7歳であった．子宮体癌の進行期は8例がI期，1例がIII期であった．予後は良好であり，2例が死亡し，1例は子宮体癌再発により，1例は異時性に発生した脳腫瘍により死亡している．

考　察

子宮体癌と他臓器癌との重複癌については，これまでにも多くの報告例があり，本邦での頻度は5.7～14.9%であった[1～10]．当科の検討では9.5%であり，過去の報告と同頻度であった．子宮体癌の治療以後に発生した癌に関しては把握しきれていない症例もあり，実際の頻度はもっと高いと思われる．

重複癌は，発生時期により同時性，異時性重複癌に分けられ，本邦での同時性重複癌の頻度は2.1～7.7%である．卵巣癌が最も多く，子宮頸癌が次いで多く，女性生殖器以外では大腸癌，乳癌，胃癌が多かった（表4）．卵巣癌との同時性重複癌

表5　子宮体癌との異時性重複癌の本邦報告例

報告者	体癌症例数	異時性重複癌症例数（頻度%）		重複癌種別症例数					
				大腸癌[a]		乳癌		胃癌	
		体癌前	体癌後	体癌前	体癌後	体癌前	体癌後	体癌前	体癌後
水内ら[1]	129	7	5	0	0	4[b]	2	0	1
古久保ら[2]	191	11	4	2	1	2	0	0	0
中山ら[3]	486	21	12	4	5	11	3	3	3
阿部ら[4]	238	3	1	1	1	2	0	0	0
市川ら[5]	147	11	4	2	1	8	2[a]	0	1[a§]
卜部ら[6]	156	2	1	0	0	2	0	0	1
嶋本ら[7]	97	2	2	0	0	2	0	0	1
後藤ら[8]	153	4	0	0	0	2[b]	0	2[b]	0
笹川ら[9]	282	20	13	3	4	14	1	1	2
坂口ら[10]	261	15[c]	1[c]	3	1	12	0	–[#]	–
自験例	313	14	9	2	4	8	2	3	1
計	2,453	110（4.4%）	52（2.1%）	17	17	67	10	10	10

[a]：大腸癌は結腸癌，直腸癌を含む．
[b]：三重複癌を1例を含む．[c]：ほかに体癌との前後不明の3例あり．
[§]：平滑筋肉腫，[#]：体癌との前後が不明な1例あり．

の場合，組織型が同じ場合は，転移癌か重複癌かが問題になる．今回の症例では，4例が子宮体部と卵巣がともに類内膜癌であったが，摘出物の肉眼所見と組織所見から重複癌と判断した．Kaneki ら[13]は，卵巣癌と子宮体癌の重複癌の鑑別診断にマイクロサテライト不安定性の検索が有用であり，その検索の結果，17例の同時性重複癌と診断された症例中12例が重複癌で，5例は子宮体癌からの転移性卵巣癌であったと報告している．

異時性・同時性重複癌の女性性器癌以外で頻度の高いものは，大腸癌，乳癌，胃癌である（表4, 5）．特に，異時性重複癌での乳癌の頻度は高く，重複癌全体の48%であり，子宮体癌前の発生が重複癌全体の42%であった．乳癌の頻度は現在増加してきており，今後さらに子宮体癌と乳癌の重複癌の頻度が増加すると思われる．

乳癌から重複癌をみた場合，乳癌術後に発生する重複癌の頻度は，三浦[14]の報告では，対側乳腺（38.1%），胃（21.5%），卵巣（7.2%），子宮頸部（7.2%），子宮体部（4.4%），結腸・直腸（4.4%）の順であり，子宮体癌は乳癌の術後に発生しやすい重複癌として知っておく必要がある．また，橋本ら[15]は，乳癌術後の婦人科検診でタモキシフェン内服群と非内服群に分けて検討した結果，それぞれ65例中2例，86例中1例の子宮体癌を発見しており，タモキシフェンの投与の有無にかかわらず，定期的な婦人科検診が必要であると思われる．

子宮体癌と大腸癌との重複癌は同時性癌では女性性器以外では最も多く（表4），異時性癌では乳癌に次いで多かった．異時性の場合，体癌の前後で頻度は変わらなかった（表5）．

遺伝性非ポリポージス大腸癌（HNPCC）家系では，大腸癌に次いで子宮体癌が高率に発生することが知られている．また，この場合の子宮体癌は大腸癌を含む重複癌を高率に発生する[16]．子宮体癌に大腸癌を合併する場合，特に家族歴の調査が重要であり，その後の経過観察も必要である．

子宮体癌は近年増加してきており，当科で扱う子宮体癌患者は10年前の約2倍になっており，それに伴い今後も重複癌が増加すると思われる．特に，子宮体癌術後に発生する頻度が高い乳癌，大腸癌に関しては，検診を受診することが重要であると思われる．

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Anti-V3 Humanized Antibody KD-247 Effectively Suppresses Ex Vivo Generation of Human Immunodeficiency Virus Type 1 and Affords Sterile Protection of Monkeys against a Heterologous Simian/Human Immunodeficiency Virus Infection

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In an accompanying report (Y. Eda, M. Takizawa, T. Murakami, H. Maeda, K. Kimachi, H. Yonemura, S. Koyanagi, K. Shiosaki, H. Higuchi, K. Makizumi, T. Nakashima, K. Osatomi, S. Tokiyoshi, S. Matsushita, N. Yamamoto, and M. Honda, J. Virol. 80:5552-5562, 2006), we discuss our production of a high-affinity humanized monoclonal antibody, KD-247, by sequential immunization with V3 peptides derived from human immunodeficiency virus type 1 (HIV-1) clade B primary isolates. Epitope mapping revealed that KD-247 recognized the Pro-Gly-Arg V3 tip sequence conserved in HIV-1 clade B isolates. In this study, we further demonstrate that in vitro, KD-247 efficiently neutralizes CXCR4- and CCR5-tropic primary HIV-1 clade B and clade B′ with matching neutralization sequence motifs but does not neutralize sequence-mismatched clade B and clade E isolates. Monkeys were provided sterile protection against heterologous simian/human immunodeficiency virus challenge by the passive transfer of a single high dose (45 mg per kg of body weight) of KD-247 and afforded partial protection by lower antibody doses (30 and 15 mg per kg). Protective neutralization endpoint titers in plasma at the time of virus challenge were 1:160 in animals passively transferred with a high dose of the antibody. The antiviral efficacy of the antibody was further confirmed by its suppression of the ex vivo generation of primary HIV-1 quasispecies in peripheral blood mononuclear cell cultures from HIV-infected individuals. Therefore, KD-247 promises to be a valuable tool not only as a passive immunization antibody for the prevention of HIV infection but also as an immunotherapy for the suppression of HIV in phenotype-matched HIV-infected individuals.

Because most primary strains of human immunodeficiency virus type 1 (HIV-1) are relatively resistant to neutralization, the specificities of antibodies that confer protective immunity against it are still not understood (22). Previously, we and others (9, 31) have reported that chimpanzees can be protected against infection with the T-cell-line-adapted strain HIV-1_{IIIB} by passive transfer of either HIV immunoglobulin (Ig) (HIVIG) or anti-HIV-1_{IIIB} V3 monoclonal antibodies (MAbs). Passive administration of the anti-HIV-1 gp41 human MAb 2F5 (24) to two chimpanzees prior to challenge with primary HIV-1_{5016} resulted in a delay in plasma viremia and reduced viral load. Since the chimpanzee model is limited by the failure of HIV-1 to induce disease in these animals, a pathogenic model was developed in monkeys using a simian/human immunodeficiency virus (SHIV) strain that is capable of inducing high plasma viremia, $CD4^+$-T-cell loss, and simian AIDS (11, 14, 15, 37). Following pathogenic SHIV 89.6P challenge, Mascola and colleagues (20) previously noted a synergistic effect with the passively transferred antibody HIVIG, a MAb against membrane-proximal external region 2F5 (27), and 2G12, a glycan-dependent MAb (41). Monkeys were afforded protective immunity against pathogenic SHIV DH12 by chimpanzee HIVIG and provided sterile protection against the challenge virus when given high-dose inoculations (27, 36). However, sterile protection was strain specific, and the antiserum did not bind a V3 loop peptide or block the interaction of gp120 with CD4. In several passive immunization studies using MAbs, the antibodies 2G12 and 2F5 as well as 4410, a MAb against membrane-proximal external region 4E10 (4), have been shown to inhibit SHIV in monkeys (2, 20, 21). Furthermore, human MAb b12, targeting the CD4-binding domain of gp120, has been reported to elicit complete protection against viral challenge (29) and partial protection against MAb 2G12 (22) in monkeys. Recently, passively transferred antibodies with 2G12, 2F5, and 4E10 were shown to delay the rebound of HIV-1 after the cessation of antiretroviral therapy, with that delay especially pronounced in acutely infected individuals.

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The in vivo effect of the neutralizing antibody cocktail was found to depend on 2G12 activity by escape mutant analysis (42).

It has been established that anti-V3 antibodies, induced by brief immunization protocols in animals, are capable of neutralizing HIV-1 in cell cultures and in animal challenge studies (13, 16, 27, 28). However, that capability has not been fully exploited because the V3 sequence is extremely diverse, and so the anti-V3 antibodies are extremely type specific and displayed little cross-reactivity. In the accompanying paper (8a), we describe how we sequentially immunized mice with V3 peptides derived from several different HIV-1 clade B field isolates. The antibody response could be traced to a tip sequence of the HIV-1 gp120 V3 domain, a relatively conserved motif (11, 18, 45). We reshaped anti-V3 MAb C25 into KD-247, a humanized MAb directed against the V3 tip motif Pro-Gly-Arg of the V3 domain. KD-247 cross-neutralized primary isolates with a matching neutralization sequence motif, suggesting that it could be used to overcome the previous limitations surrounding anti-V3 neutralizing antibody production by active immunization strategies.

In this study, we show that the humanized MAb KD-247 is suitable not only for use as a passive immunization antibody for the prevention of immunodeficiency virus infection but also to passively transfer antibodies for immunotherapy. Using 18 primary HIV-1 isolates, we evaluate the neutralizing capacity of KD-247. We also assess its efficacy against ex vivo generation of HIV from the peripheral blood mononuclear cells (PBMCs) of four HIV-infected individuals. Finally, we examine whether KD-247 can suppress HIV-1 replication in monkeys.

MATERIALS AND METHODS

Passive transfer of KD-247 to monkeys followed by pathogenic virus challenge. All animals used in this study were mature, cycling, male cynomolgus monkeys (*Macaca fascicularis*) from the Tsukuba Primate Center, National Institute of Infectious Diseases (NIID), Japan. They were free of known simian retroviruses, herpesviruses, bacteria, and parasites. They were housed in accordance with the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science under the Japanese Law Concerning the Protection and Management of Animals (1, 38) and were maintained in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of NIID, Japan. Once approved by an institutional committee for biosafety level 3 experiments, these studies were conducted at the Tsukuba Primate Center, NIID, Japan, in accordance with the requirements specifically stated in the laboratory biosafety manual of the World Health Organization (44a).

The pathogenic SHIV strain C2/1 is an SHIV strain 89.6 variant isolated by in vivo passage in cynomolgus monkeys (37). The original SHIV 89.6 strain was kindly provided by Y. Lu at the Harvard AIDS Institute (Boston, MA) (19, 32). Virus stocks of SHIV C2/1 were stored at −125°C and thawed just prior to use. The challenge stock was provided by K. Shinohara of the National Institute of Infectious Diseases, Tokyo, Japan. Cynomolgus monkeys injected intravenously with SHIV C2/1 showed high levels of viremia and marked $CD4^+$-T-cell depletion within 2 weeks after inoculation (1, 34, 35, 37). Naïve monkeys were intravenously administered 0, 15, 30, or 45 mg/kg of KD-247 along with either 45 mg/kg of purified normal human immunoglobulin (Nihon Pharmaceutical Co., Tokyo, Japan) or saline. Twenty-four hours after antibody transfer, the animals were intravenously challenged with 20 50% tissue culture infective doses ($TCID_{50}$s) of SHIV C2/1.

In vitro virus neutralization assays. The primary clinical isolate $HIV\text{-}1_{MNp}$ was kindly provided by J. Sullivan of the University of Massachusetts Medical School, Worcester, MA. The virus was confirmed to be neutralization resistant (5). Laboratory-adapted $HIV\text{-}1_{89.6}$ and $HIV\text{-}1_{MN}$ were obtained from the AIDS Research and Reference Reagent Program, National Institutes of Health, Rockville, MD. GHOST cell neutralization assays were performed as described previously (5, 38). Briefly, GHOST cells expressing either CXCR4 or CCR5 coreceptors were used as targets of HIV-1 infection. The cells were then analyzed by FACSCalibur flow cytometry (Becton Dickinson, San Jose, CA). The same concentration of either purified normal human immunoglobulin consisting primarily of the IgG1 subclass (Nihon Pharmaceutical Co.) or saline was used as control.

Neutralization activities in monkey plasma were assayed by detecting the neutralizing titers in the assay measuring 100% neutralization against the challenge virus as described previously by Nishimura et al. (26). In brief, plasma samples were serially diluted and incubated with 100 $TCID_{50}$s of challenge virus, and M8166 cells were then incubated as previously described (26). The neutralization was expressed as the percent inhibition of simian immunodeficiency virus p27 antigen production in the culture supernatants (38, 39). Normal monkey plasma was used as a control.

PBMC-based virus neutralization assay. $HIV\text{-}1_{MN}$ (H9/HTLV-III MN) was kindly provided by the AIDS Research and Reference Reagent Program, National Institutes of Health, Rockville, MD (45). The WHO primary isolates 92TH002, 92TH022, 92TH023 (all clade E), and 92TH014 (clade B′) were used as virus stocks (12). The primary isolates $HIV\text{-}1_{JR\text{-}CSF}$ and the CS and JCI series of HIV-1 isolates were provided by Y. Koyanagi (40) and Y. Okamoto (27). In vitro virus neutralization assays were performed as previously described (7, 12). Neutralization titers are expressed as either the concentration of serum IgG antibody or the reciprocal of the serum dilution that yielded a 50% (50% inhibitory concentration [IC_{50}]) or 90% (IC_{90}) reduction in HIV-1 p24 production over that seen in controls using purified serum IgG from healthy individuals or preimmune mouse sera.

Ex vivo virus neutralization assays. The PBMCs of patients infected with HIV-1 were depleted of $CD8^+$ cells by magnetic separation using polystyrene beads coated with anti-CD8 MAb (Dynabeads M-450 CD8; Dynal, Oslo, Norway). The negatively selected cells were stimulated with OKT3 antibody (1 μg/ml; Jannsen-Kyowa, Tokyo, Japan) and subsequently cultured in the presence of interleukin-2 (20 U/ml; Boehringer, Mannheim, Germany) together with KD-247 (60 and 240 μg/ml). The amount of HIV-1 p24 antigen in the supernatant was determined by enzyme-linked immunosorbent assay (ELISA) (Dainabot, Tokyo, Japan). Approval by the ethical committee and written informed consent from all the human subjects were obtained according to the guidelines of the Ministry of Health, Labor, and Welfare, Japan, and to those of the Kumamoto University Medical School, Kumamoto, Japan.

Competitive PCR quantitation of SHIV RNA in plasma. Quantitative competitive reverse transcription-PCR was performed as described previously by Piatak et al. (30), with both the substitution of a different competitor RNA and a different DNA template (35). The detection limit of this assay was 500 RNA copies/ml in monkey plasma.

Flow cytometric evaluation of cell surface antigen expression and absolute cell count. Mouse MAbs conjugated with either fluorescein isothiocyanate, phycoerythrin (PE), PE-Cy5, or peridinin chlorophyll protein were used in flow cytometric analyses to detect cellular expression of monkey CD3 (NF-18; BioSource International Inc., Camarillo, CA), human CD4 (Nu-TH/I; Nichirei Co., Tokyo, Japan), CD8 (SK-1; Becton Dickinson & Co., San Jose, CA), and CD95 (CH11 and 7C11; Becton Dickinson) (30). To determine absolute cell counts, samples of whole blood were analyzed following the addition of fluorescein isothiocyanate-conjugated anti-CD3 (BioSource), PE-conjugated anti-CD4 (Becton Dickinson), and peridinin chlorophyll protein-conjugated anti-CD8 (Becton Dickinson) MAbs as previously described (35).

Plasma concentration of KD-247. HIV-1 V3 peptide-based ELISA was used for quantification of KD-247 antibody. In brief, 96-well ELISA plates (Maxisorp; Nunc A/S, Roskilde, Denmark) were coated with 100 μl of a KD-247 antigen peptide (SP1 [YNKRKRIHIGPGRAFYTTKNC]) per well in 50 mM carbonate buffer (pH 9.3) at 1 μg/ml overnight at 4°C. KD-247 was diluted to concentrations ranging from 2.5 to 40 ng/ml as a reference. Bound KD-247 was detected with a peroxidase-conjugated anti-human IgG MAb (in-house preparation; The Chemo-Sero-Therapeutic Research Institute). The concentrations of KD-247 in the plasma of monkeys were determined using a calibration curve (SOFTmax; Molecular Devices Co., Menlo Park, CA).

Statistical analysis. The plasma concentrations at various data points postdose were applied to a two-compartment model using an automatic pharmacokinetic analysis program (nonlinear least-squares method), and pharmacokinetic parameters were calculated.

RESULTS

Neutralization ability of the humanized antibody KD-247 against a panel of primary isolates as determined by a PBMC-based study. In the initial series of the study, we showed that

TABLE 1. PBMC-based neutralization of primary and laboratory isolates by KD-247[a]

Isolate	Env V3 sequence[b]		GHOST cell	KD-247		447-52D IC_{50}[c]
				IC_{90}	IC_{50}	
Laboratory isolates, clade B						
$HIV\text{-}1_{MN}$	CTRPNYNKRKRIHI	GPGRAFYTTKNIIGTIRQAHC	X4	1	0.1	0.1
$HIV\text{-}1_{SF2}$	-----N-T--G---	-------A-EK-V-D------	X4	5	1.0	1.0
$HIV\text{-}1_{89.6}$	-----N-T-R-LS-	-------ARR----D------	R5/X4	2.5	0.2	>10
Primary isolates, clade B						
$HIV\text{-}1_{JR\text{-}CSF}$	----SN-K--S---	---------GE---D------	R5	5	0.4	>10
$HIV\text{-}1_{CS2\text{-}2}$	-----N-T--S--M	---K-----GD---N----Y-	R5	>50	>50	ND
$HIV\text{-}1_{CS3\text{-}5}$	---I-N-T--S---	-------A-GE---N-K----	R5	10	1.4	ND
$HIV\text{-}1_{CS4\text{-}4}$	-I---N-T--G---	-L-- WK--A-G--N------	R5/X4	>50	>50	ND
$HIV\text{-}1_{CS6\text{-}6}$	--G--N-T--S-R-QR	------V-IGK --NM-----	R5	>50	>50	ND
$HIV\text{-}1_{CS6\text{-}8}$	-I---N-T--G---	-------A-D----N------	R5	8	1.2	ND
$HIV\text{-}1_{JCI\text{-}1}$	----HKTI------	----------Q-E-N-----	X4	5	0.4	ND
$HIV\text{-}1_{JCI\text{-}2}$	----SN-T-R----	---------RQ-R-D-----	X4	4	0.2	ND
$HIV\text{-}1_{JCI\text{-}3}$	-----N-I--H---	---------RG -RD--K---	R5	10	0.6	ND
$HIV\text{-}1_{JCI\text{-}5}$	-------T--G---	------V---G -RD--K---	X4	4	0.2	ND
$HIV\text{-}1_{JCI\text{-}6}$	----SN-T-R----	-----S--A-Q-RGD------	X4	6	0.7	ND
$HIV\text{-}1_{JCI\text{-}9}$	-------T--G---	------V---G -RD--K---	R5	21	1.6	ND
$HIV\text{-}1_{JCI\text{-}11}$	-------TS-G-R-	-------ASER -RD--K---	R5	34	3.2	ND
$HIV\text{-}1_{JCI\text{-}22}$	-----N-I--H---	---------RG -RD--K---	R5	12	1.2	ND
Primary isolates, clade B′						
$HIV\text{-}1_{92TH014}$	-----N-T--S-PL	-----W---GQ---D------	R5	8	0.9	>1.5
Primary isolates, clade E						
$HIV\text{-}1_{92TH002}$	----SN-T-TS-T-	---QV--R-GD---D--K-Y-	R5	>50	>50	ND
$HIV\text{-}1_{92TH022}$	----SN-T-TS-T-	---QV--R-GD---D--K-Y-	R5	>50	>50	>10
$HIV\text{-}1_{92TH023}$	----SN-T-TS-N-	---QV--R-GD---D--K-Y-	R5	>50	>50	ND
SHIV-B						
SHIV 89.6PD	-----N-T-R-LS-	-------ARR----D------	R5/X4	5	0.5	ND
SHIV C2/1	-----N-T-E-LS-	-------ARR----D------	R5/X4	5	0.5	ND

[a] The HIV-1 sequences were confirmed by proviral DNA sequencing of virus-infected cells.
[b] Dashes indicate sequence homology to $HIV\text{-}1_{MN}$, and spaces represent the presence of a deletion.
[c] ND, not done.

sequential immunization with synthetic V3 peptides from representatives of primary HIV-1 clade B isolates generated cross-reactive antisera and produced a high-affinity humanized MAb, KD-247, directed against the tip of the HIV-1 V3 domain, PGR. Furthermore, the humanized antibody more effectively neutralized several primary isolates of HIV-1 clade B than did previously reported neutralization antibodies (8a, 10, 23, 27). To further analyze the divergence of the cross-neutralization ability of the antibody by a PBMC-based HIV-1 neutralization assay, we used a panel of a total of 23 immunodeficiency viruses: 18 primary isolates of HIV-1 clade B, clade B′, and clade E viruses; 3 laboratory HIV-1 clade B viruses; and 2 highly pathogenic SHIVs (Table 1). The KD-247 antibody effectively neutralized $HIV\text{-}1_{MN}$, $HIV\text{-}1_{SF2}$, and $HIV\text{-}1_{89.6}$, containing the consensus V3 sequence of HIV-1 clade B, IGPGR AFY, with an IC_{90} and IC_{50} from 1 to 5 and from 0.1 to 1.0 μg/ml, respectively (Table 1, laboratory isolates, clade B). We next sought to assess whether the neutralization of primary isolates by KD-247 required a matching neutralization sequence motif. As expected, KD-247 effectively neutralized primary CCR5-tropic clade B and B′ isolates (IC_{90} and IC_{50} from 5 to 34 and from 0.4 to 3.2 μg/ml, respectively) and all four of the CXCR4-tropic clade B isolates (IC_{90} and IC_{50} from 4 to 6 and from 0.2 to 0.7 μg/ml, respectively) with matching IGPGR or V3 tip sequences. Thus, CCR5-tropic isolates with an IC_{90} of a mean concentration of neutralization antibody of 13.5 μg/ml were more than 2.8 times less sensitive to the neutralization by KD-247 than primary CXCR4-tropic isolates with a mean IC_{90} of 4.8 μg/ml. In contrast, the neutralization-resistant virus CS2-2 did not match the neutralization sequence motif, and the CS6-6 virus showed a QR insertion in the V3 tip sequence. The HIV-1 isolates containing a glutamine (Q) residue at position 20 in the V3 region, such as those of subtype E, were also resistant to neutralization by KD-247. Therefore, KD-247 effectively neutralizes both the CCR5- and CXCR4-tropic primary isolates with matching neutralization motifs.

Ex vivo suppressive effects of KD-247 on the generation of HIV-1 quasispecies from PBMCs of HIV-infected individuals. To fully assess the antiviral efficacy of KD-247, we next sought to determine whether it would suppress the generation of HIV-1 from PBMCs of HIV-infected individuals and whether it would do so as efficiently as an established anti-V3 humanized antibody, Cβ1 (23). As shown in Table 2, we investigated the effect of KD-247 at concentrations of 60 and 240 μg/ml on the ex vivo generation of HIV-1 using $CD8^+$-T-cell-depleted PBMC cultures from four Japanese individuals infected with HIV-1 clade B (Env V3 sequence in Table 2). In the presence of KD-247 at concentrations of 60 and 240 μg/ml, the gener-

TABLE 2. Ex vivo neutralizing activity of KD-247 against HIV-1 present in PBMC cultures established using cells from HIV-infected individuals[a]

Patient	HIV-1 Env V3 sequence (no. of clones)	PBMCs, (no. of cells/well)	KD-247 (μg/ml)	p24 (log_{10} pg/ml)
KU008	CTRPHNNTRKSIHIGPGRAFYATGDIIGNIRQAHC (3)	6.5×10^5	0	3.93
	----------E---D--R-- (2)		60	0.37
	----------E---D----- (1)		240	0.08
	----------D----- (1)			
KU045	CTRPNNNTRKGIHIGPGRAFYGTDIVGDIRQAHC (5)	7.3×10^5	0	3.70
	----------E-T-N----Y- (2)		60	0.88
	----------N----- (1)		240	0.56
KU037	CTRPNNNTRKSIPIGPGRAFYATGDIIGDIRKAHC (3)	1.3×10^6	0	3.81
	-------I---------- (1)		60	3.86
	-I------G---------- (1)		240	0.25
KU040	CTRPNNNTRKSVHIGPGRAWYATGEIIGNIRQAHC (2)	8.0×10^5	0	4.12
	----------A----F---------- (1)		60	2.34
	----------I--------H---------- (1)		240	2.62
	---H--------I-L---G--H---D--------- (1)			

[a] Ex vivo neutralization activity was directly detected by using $CD8^+$ cell-depleted PBMCs from HIV-infected individuals as described in Materials and Methods.
[b] The number of analyzed DNA clones from each patient is indicated in parentheses. Dashes indicate sequences identical to those of the upper major clone from each patient.

ation of viruses from PBMCs of KU008 was reduced in a dose-dependent manner, with 3.56- and 3.85-log reductions in the culture supernatants, respectively; reductions of 2.82 and 3.14 logs of virus generation from PBMCs of KU045 were also detected in the presence of 60 and 240 μg/ml of KD-247, respectively, KU037 showed a reduction of 3.56 logs at only 240 μg/ml. However, KU040 showed no dose-dependent suppressive effects of virus generation by KD-247. When the irrelevant antibodies of Cβ1 and normal serum IgG were added to cell cultures, they showed no suppressive effects on virus generation (data not shown). These results demonstrate that KD-247 effectively neutralizes nonpassage viruses generated in the primary culture of PBMCs from individuals infected with HIV-1 clade B with neutralization sequence motifs matching that of the quasispecies, IGPGR.

Induction of complete protection of monkeys against a highly pathogenic SHIV strain by a single passive transfer of a high dose of KD-247. PBMCs from 12 juvenile male cynomolgus monkeys were first evaluated in vitro to establish their susceptibility to infection with the SHIV C2/1 challenge stock in standard viral infectivity assays (35, 37) (data not shown). Challenge virus SHIV C2/1 originated from SHIV 89.6 but did share an identical envelope sequence with the parental strain, $HIV\text{-}1_{89.6}$, and showed 17 nucleotide mutations with amino acid changes (1, 34). The neutralization sensitivity of SHIV C2/1 to KD-247 was found to be similar to that of $HIV\text{-}1_{89.6}$, with an IC_{90} and IC_{50} of 5 and 0.5 μg/ml in human PBMC-based neutralization assays, respectively (Table 1, laboratory isolates, clade B and SHIV-B), suggesting that the neutralization potency of KD-247 in vitro might be sufficient to warrant passive transfer experiments.

Of the 12 monkeys, 5 were inoculated with KD-247, 2 were inoculated with control normal human IgG (NHIgG) (45 mg/kg), and the remaining 5 were given saline alone. Of the five animals receiving KD-247, two were given a dose of 45 mg/kg, two received 30 mg/kg, and one received 15 mg/kg. Twenty-four hours after antibody transfer, all 12 monkeys were given an intravenous challenge of 20 $TCID_{50}$s/ml SHIV (Fig. 1). At the time of viral challenge, the plasma concentrations of KD-247 were 151, 443, 496, 866, and 678 μg/ml of the antibody in immune sera from monkeys 3968, 3969, 3972, 4092, and 4099, respectively (Fig. 1a). The area under the plasma concentration time curve (AUC) values for monkeys 3968, 3969, 3972, 4092, and 4099 were calculated from the antibody concentration data to be 1.8, 3.5, 5.0, 6.5, and 5.6 mg · day/ml, respectively.

The percentage of $CD4^+$ T cells and the levels of plasma viremia were also monitored after SHIV challenge (Fig. 1b and c). All monkeys that were intravenously inoculated with normal human IgG or saline showed a loss of $CD4^+$ T cells within 7 days of viral challenge, accompanied by plasma viremia reaching 10^7 to 10^8 viral RNA copies/ml (data from the five control monkeys that received saline alone are not shown). Of the two control monkeys that received 45 mg/kg of NHIgG, both seroconverted against SHIV p27 antigen (monkeys 3967 and 3974) (Fig. 1d). At autopsy, all control monkeys showed $CD4^+$-T-cell depletion in lymphoid organs, a finding consistent with our previous observations using this model (35, 37).

Both monkeys that received a single high dose of 45 mg of KD-247 per kg of body weight prior to SHIV challenge were completely protected from viral challenge, maintaining stable $CD4^+$-T-cell counts and not seroconverting or exhibiting plasma viremia (Fig. 1b to e, monkeys 4092 and 4099, indicated by red lines and red characters). When evaluated at autopsy using PCR for SHIV *gag* proviral DNA, their tissues showed no sign of infection (data not shown). The titers in plasma resulting from 100% in vitro neutralization against 100 $TCID_{50}$s of the challenge virus at the time of virus challenge were 1:160 in both monkeys 4092 and 4099. The titers in partially protected monkeys 3969 and 3972 were 1:40 and 1:80, respectively. No neutralization activity of less than 1:10 was measured in the animals receiving 45 mg/kg of NHIgG (monkeys 3967 and 3974). Thus, although the highest titers of neutralization activities were detected in plasma from protected animals, the neutralization activity was high even in animals with only partial protection.

Administration of lower doses of KD-247, 30 mg/kg to two monkeys (monkeys 3969 and 3972, indicated by blue lines and

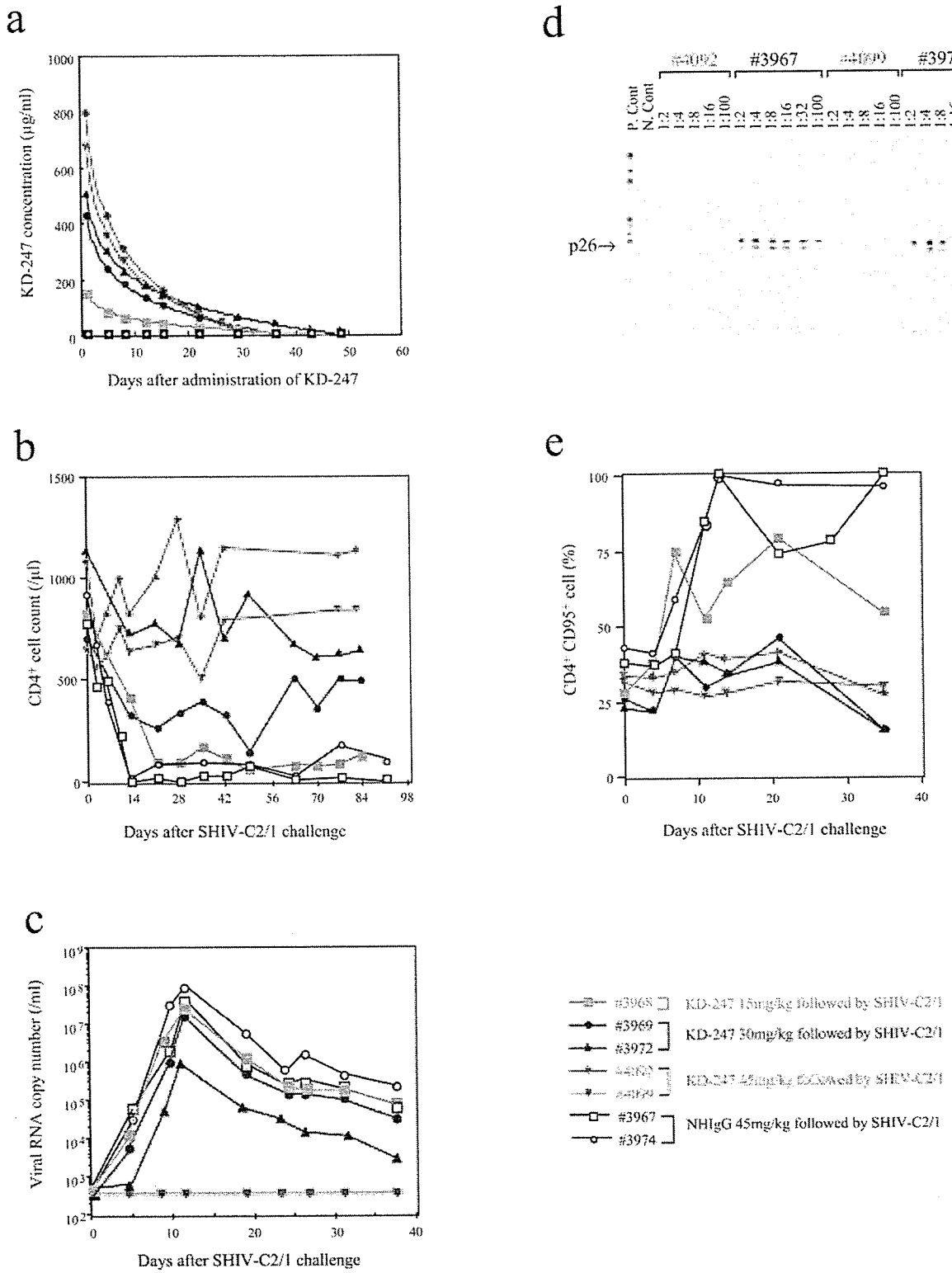

FIG. 1. KD-247 efficiently protects monkeys from pathogenic virus challenge. A total of 12 cynomolgus monkeys were used for virus challenge studies with SHIV C2/1. In the first group, five monkeys were intravenously inoculated with various doses of KD-247, followed by 20 $TCID_{50}$s of SHIV C2/1 challenge 24 h after antibody transfer. Monkeys in the second and third groups were injected prior to virus challenge with either 45 mg/kg of normal human immunoglobulin (two monkeys) or saline alone (five monkeys). The following parameters were measured in monkeys given KD-247: (a) concentration of KD-247 in plasma following passive transfer, (b) $CD4^+$-T-cell counts, (c) plasma viremia, (d) Western blot analysis using an HIV-2 Western blot kit (Diagnostics Pasteur, Marnes-La-Coquette, France) (6) of serum samples obtained at autopsy from monkeys given a single high dose (45 mg/kg) of KD-247 (monkeys 4092 and 4099) or NHIgG controls (monkeys 3967 and 3974), and (e) CD95 antigen expression on PBMCs from monkeys challenged with SHIV.

blue characters in Fig. 1) and 15 mg/kg to one monkey (monkey 3968, indicated by green lines and green characters in Fig. 1), afforded partial protection from SHIV infection. Monkey 3972 (Fig. 1, closed triangle with blue line) showed better partial protection than monkey 3969, which received 30 mg/kg of antibody. That superior degree of partial protection may be related to better blood concentration of the antibody and to better AUC values. All three monkeys described above seroconverted against SHIV p27 antigen (data not shown), but their loss of $CD4^+$ T cells seemed to be inversely proportional to the plasma concentration of KD-247 (Fig. 1a and b). Although the $CD4^+$-T-cell decline indicated minimal protection in the monkey given 15 mg/kg of KD-247 (monkey 3968) (Fig. 1b), CD95 antigen expression, a marker for cell stimulation, was significantly lowered in this animal and completely inhibited in the other four monkeys receiving KD-247 (Fig. 1e), suggesting that KD-247 significantly suppressed PBMC stimulation by the virus challenge in these animals (monkeys 3969, 3972, 4092, and 4099).

These results therefore demonstrate that KD-247 efficiently neutralizes primary HIV isolates regardless of cell tropism. Furthermore, passive immunization with a single dose of 45 mg of antibodies per kg of body weight 24 h prior to viral challenge completely protected animals from viral challenge, showing that at high concentrations, KD-247 lowers the viral load and induces sterilizing immunity in the monkey model.

DISCUSSION

In this study, KD-247 proved an effective antiviral agent for the targeting of phenotype-matched viruses, one capable of both in vitro neutralization of primary isolates and in vivo passive transfer of the antibody as well as of suppressive effects against ex vivo generation of HIV from HIV-infected individuals. Although it has already been established that brief immunizations with a V3 peptide can elicit neutralizing antibodies to homologues of the CXCR4-tropic virus, the limitations of anti-V3 antibodies have been known for over a decade (8, 13, 16, 28). Also, at reasonable IC_{50}s, the anti-V3 antibodies did not neutralize CCR5-tropic strains. In the accompanying paper (8a), we described the derivation of a humanized MAb, KD-247, that was produced by sequential immunization using six different HIV-1 Env V3 peptides derived from HIV-1 clade B field isolates. We suggested that KD-247 could potentially overcome the previous limitations to immunologically exploiting the anti-V3 antibody induced by brief immunization protocols, i.e., its extraordinary sequence variability and the associated isolate specificity of anti-V3 antibodies (27, 38). The findings of our current study suggest that KD-247 may curb the spread of viral infection and reduce viral loads in HIV-infected individuals who have been determined to share the V3 tip sequence of the virus by virus neutralization phenotype-matching analysis.

In vitro, KD-247 has potent neutralizing activity against a variety of primary HIV-1 clade B isolates, including CCR5-tropic viruses, at low concentrations. We found that KD-247 neutralized a variety of clade B primary viruses containing IGPGR V3 sequences, although its neutralization ability was affected by some of the surrounding amino acids of the V3 tip region, as discussed in the accompanying paper (8a). Based upon these results, we should be able to predict the neutralization ability of KD-247 by prior sequencing of the HIV-1 Env V3 region of the target virus. Using the previously published sequences found in the Los Alamos HIV-1 sequence database, we determined that the IGPGRA sequence is present in the majority of HIV-1 clade B isolates (45) to which KD-247 would be expected to have cross-neutralization activity. Moreover, KD-247 significantly curbed the generation of primary HIV-1 quasispecies in ex vivo cultures of $CD8^+$-T-cell-depleted PBMCs from seropositive individuals. However, as described above, the major limitation of KD-247 as an antiviral agent is its inability to neutralize variants expressing amino acid alterations in the binding site PGR motif and additional amino acids.

What are the properties that make KD-247 an effective neutralizer of CCR5-tropic viruses? First, the site-specific binding of KD-247 to epitopes on the virus envelope glycoprotein seems to be key to its virus neutralization ability. Indeed, the results of the Pepscan analysis reported in the accompanying paper suggest that KD-247 can react with core V3 sequences from various HIV-1 clade B isolates (8a). The shortest peptide that was reactive with KD-247 was IGPGR, but that epitope was stabilized by the addition of one or more amino acids. Furthermore, IGPGRA and GPGRAF sequences occur in the majority of HIV-1 isolates from donors in the United States (17). The results of Pepscan with replacement peptides also suggest that KD-247 has broad binding activity to HIV-1. While the number of amino acid substitutions tolerated in the central PGR sequence of the V3 tip peptide was small, replacement of amino acids in the flanking region was relatively permissible. Second, ex vivo neutralization assays using patient-derived isolates containing APGR and GPGG sequences in the V3 tip showed incomplete neutralization (Table 2, KU040). Thus, KD-247 would be expected to bind with HIV-1 quasispecies having a recognition sequence similar to the neutralization phenotype. Third, as the accompanying paper demonstrates, high-affinity antibody binding is apparently required for neutralization, because the kinetic parameters of KD-247 were identified to be fast on and slow off rates, similar to those of a type-specific MAb, Rμ5.5, although the equilibrium dissociation constant value of KD-247 for binding to a control SP1 peptide was higher than that of Rμ5.5 (8a). This is a reasonable assumption, since the epitope of KD-247 (IGPGR) is shorter than that of Rμ5.5 (IHIGPGRAFYT). The high association rate of KD-247 might be responsible for exerting the observed cross-neutralization activity against various primary isolates. These results are consistent with the hypothesis that virus neutralization can be explained by the kinetic parameters of antibody binding.

Most recent passive transfer studies with monoclonal antibodies used common combinations of broadly cross-reactive human MAbs capable of neutralizing primary HIV-1 isolates. In monkeys, human MAbs b12 (29) and 2G12 (20) were shown to induce complete and partial protection, respectively, against viral challenges. In contrast, the MAb chosen for this study, KD-247, is a humanized antibody induced by sequential immunization with a set of V3 peptides from primary isolates. Because the KD-247 IC_{90} value from an in vitro neutralization assay in our study, 5.0 μg/ml of the antibody, approximates that obtained by a single antibody, b12 (3), and a combination of

the two MAbs 2F5 and 2G12 or a triple combination of HIVIG, 2F5, and 2G12, as previously reported (41, 43), we postulated that KD-247 was sufficiently potent to achieve protection of monkeys against a pathogenic SHIV challenge. Since our previous experience (9) has taught us to expect approximately 500 to 1,000 μg/ml in sera from monkeys passively immunized with 30 to 45 mg of antibody per kg of body weight, the potency of KD-247 should prove sufficient for passive transfer experiments of effective antibodies in animals in vivo. We also expected that a single passive transfer of KD-247 via inoculation with 15 and 30 mg of antibody would result in approximately 150 to 500 μg/ml of plasma concentration at the time of viral challenge. As expected, we found an AUC value of 1.8 to 5.0 mg · day/ml. Consequently, we found that animals passively immunized with 45 mg/kg of KD-247 showed 678 and 866 μg/ml of KD-247 in plasma at the time of viral challenge and an AUC value of 5.6 and 6.5 mg · day/ml. Those animals were provided sterile protection against intravenous challenge with the pathogenic virus SHIV C2/1. The protective endpoint titers of neutralization antibodies in plasma at the time of virus inoculation were 1:160 in both animals that elicited sterile immunity, and a high titer of neutralization activity in plasma was similarly detected in completely protected monkeys, as described previously by Nishimura et al. (26) and Parren et al. (29). Thus, the high titers of neutralization activity in plasma confer sterile protection against viral challenge in the passively immunized animals with neutralizing antibodies. Furthermore, the pharmacokinetic information consisting of the plasma concentration of the neutralizing antibodies at the time of viral challenge and the AUC value may be closely related to the ability of the antibody to provide sterile protection against viral challenge. Since those protected macaques demonstrated the inhibition of $CD4^+$ cell loss, the pharmacokinetic properties of KD-247 may also be closely associated with the inhibition of $CD4^+$ cell decline in the peripheral circulation of the challenged monkeys.

In this study, we also detected lower viremia with lesser $CD4^+$ cell decline in animals that were inoculated with intermediate doses of antibody. However, we noted that the lesser doses of the antibody provided complete protection against enhanced rates of the $CD4^+$ $CD95^+$ cell subpopulation in the peripheral circulation of the challenged animals, suggesting that the reshaping MAb might be able to control the activation of peripheral $CD4^+$ T cells in animals by its passive transfer. Although the number of monkeys enrolled in this study was limited, it remains noteworthy that a single inoculation with KD-247, even at a suboptimal dose for viral protection, appeared to be effective for maintaining $CD4^+$ T cells in monkeys inoculated with virus. Since it has been previously reported that the limited effect of neutralizing antibody may be related to the rapid appearance of an escape mutant in infected individuals, high titers of neutralization activity should be generated in the passively immunized animals (25, 33, 44). In our preliminary study, we isolated the escape mutant from the neutralization resistance virus $HIV\text{-}1_{JR\text{-}FL}$ in the presence of KD-247: at passage 8 of the culture in the presence of 1,000 μg/ml KD-247, one amino acid substitution, GPGR to GPER, was identified in the V3 tip (K. Yoshimura et al., unpublished results). Collectively, these results suggest that KD-247 shows clinical promise both for passive immunization and as a strategy for preventing viral spread in phenotype-matched HIV-infected individuals.

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The incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men

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Abstract

Recent data from Hiby (2004) have suggested that a combination of maternal killer immunoglobulin receptor (KIR) AA genotype and fetal HLA-C2 genotype increases the risk of pre-eclampsia. Different human populations have a reciprocal relationship between KIR AA frequency and HLA-C2 frequency. Japanese people have highest frequency of KIR-AA alleles and lowest frequency of HLA-C2 alleles. However, Caucasians have a moderate frequency of KIR-AA and HLA-C2 alleles. If this hypothesis is correct, the incidence of pre-eclampsia among couples consisting of Japanese women and Caucasian men should be higher than that among couples consisting of Japanese women and Japanese men. Therefore, we investigated the incidence of pre-eclampsia among 324 couples consisting of Japanese women and Caucasian men. The incidence of pre-eclampsia in these couples consisting of Japanese women and Caucasian men was similar to that in Japanese women and Japanese men. Our data do not support that of Hiby et al. [Hiby, S.E., Walker, J.J., O'Shaughnessy, K.M., Redman, C.W.G., Carrington, M., Trowsdale, I., Moffett, A., 2004. Combinations of maternal KIR and fetal HLA-C genes influence the risk of pre-eclampsia and reproductive success. J. Exp. Med. 200, 957–965], although we did not check the haplotypes for HLA-C and KIR.

Keywords: Etiology; HLA-C; KIR; Pre-eclampsia; Human population

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1. Introduction

A number of hypotheses on the etiology of pre-eclampsia have been reported (Roberts et al., 1989; Arngrimsson et al., 1990; Perry and Martin, 1992; Meekins et al., 1994; Robillard et al., 1994; Zhou et al., 1997; Dekker et al., 1998; Redman et al., 1999; Koelman et al., 2000; Saito and Sakai, 2003). One commonly discussed hypothesis is the immunogenetic maladaption hypothesis (Dekker et al., 1998; Robillard et al., 2002; Koelman et al., 2000; Saito and Sakai, 2003; Chaouat et al., 2005). Immune recognition of fetal (paternal) antigens is suggested by the increased risk of pre-eclampsia in first pregnancies (MacGillivray, 1983; Skjaerven et al., 2002) and in multiparous women after changing partners (Robillard et al., 1999; Li and Wi, 2000; Trogstad et al., 2001). There is also an increased risk in women who have received donated gametes, such as artificial donor insemination (AID) (Hoy et al., 1999), oocyte donation and embryo donation (Soderstrom-Anttila et al., 1998). These findings suggest that maternal tolerance to paternal antigens is important for the maintenance of pregnancy, and immunogenetic maladaptation of tolerance system might induce pre-eclampsia. Extravillous trophoblasts (EVT) express four unique class I MHC molecules: HLA-G, HLA-E, HLA-F and HLA-C (Kovats et al., 1990; Ishitani et al., 2003; King et al., 1996). Only HLA-C is polymorphic, so paternal HLA-C on EVT can be recognized by killer immunoglobulin receptors (KIR) on maternal NK cells (Moffett-King, 2002). Recently, Hiby et al. (2004) reported interesting data showing that the combination of maternal KIR-AA, which has no activating receptors, and the fetal HLA-C2 group is associated with pre-eclampsia. They showed also that additional activating KIRs decrease the incidence of KIRs pre-eclampsia when the fetus has an HLA-C2 allele.

Recent data demonstrate that populations with a high KIR-AA genotype frequency have a low frequency of HLA-C2 alleles and vice versa (Williams et al., 2002; Yawata et al., 2002; Norman et al., 2001; Crum et al., 2000; Cook et al., 2003; Toneva et al., 2001; Rajalingam et al., 2002; Whang et al., 2003; Wang et al., 1997). Hiby et al. (2004) hypothesized that the KIR-AA/HLA-C2 combination in a given population would be selected against by deleterious effects such as pre-eclampsia. Japanese people have the highest frequency of KIR-AA genotype at around 60%, and the lowest frequency of HLA-C2 genotype at around 9% (Yawata et al., 2002). Conversely, Australian aborigines and New India have the lowest frequency of KIR-AA genotype and highest frequency of HLA-C2 genotype (Norman et al., 2001; Cook et al., 2003; Rajalingam et al., 2002). If the hypothesis of Hiby et al. (2004) is correct, the incidence of pre-eclampsia in couples consisting of Japanese women and Australian aborigine or New India men should be high. However, such couples are very rare. On the other hand, the number of couples consisting of Japanese women and Caucasian men has been increasing. Caucasians have moderately high frequencies of KIR-AA genotype and HLA-C2 genotype. The frequency ratio of the HLA-C2 genotype is 30–35%, which is three to four times higher than that in Japanese (Williams et al., 2002; Hiby et al., 2004). Therefore, we investigated the frequency of pre-eclampsia in couples consisting of Japanese women and Caucasian men.